THE METABOLISM OF CALCIUM, PHOSPHORUS, MAGNESIUM, COPPER, ZINC AND POTASSIUM IN LAMBS AS RELATED TO DIETARY CALCIUM LEVEL

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CHAPTER I

INTRODUCTION

The ruminant animal is anatomically and physiologically suited for consumption of large quantities of herbaceous materials. These materials often contain relatively high levels of calcium. It has been established that calcium to phosphorus ratios of 1:1 to 7:1, with adequate phosphorus, do not cause significant differences in calf growth (Dowe, Matsushima and Arthaud, 1957; Wise, Ordoveza and Barrick, 1963). Ratios above these levels tend to result in a depression of growth.

Although the effects of high dietary levels of calcium, i.e., those levels above the normal requirement, have been evaluated in animal performance experiments, little or no work has been concerned with the effects of high dietary levels of calcium upon the metabolism of other minerals. In order to effectively establish optimal calcium requirements, the relationship and effect of dietary level of calcium upon other mineral elements must be elucidated. This study was conducted to evaluate the effect of the dietary level of calcium upon the metabolism of calcium, phosphorus, magnesium, copper, zinc and potassium in lambs fed a purified diet. Only those dietary levels of calcium above the normal ruminant requirement were evaluated.

CHAPTER II

REVIEW OF LITERATURE

Introduction

In order to effectively study calcium metabolism and how the level of dietary calcium affects the metabolism of other minerals, it is necessary to review the factors involved in calcium homeostasis. The factors involved in calcium regulation are very complex and involve many interrelationships. However, the role of two hormones, parathyroid hormone and calcitonin, and the role of vitamin D in calcium regulation can be discussed somewhat independently.

The following review will include a brief discussion of the role of parathyroid hormone, calcitonin and vitamin D. With this foundation, nutritional aspects of calcium metabolism and its relationship to the metabolism of other minerals will be discussed. Particular reference will be made to dietary levels of calcium considered to be in excess of the normal ruminant requirement.

Parathyroid Hormone

Parathyroid hormone is secreted by the parathyroid glands in response to a decline in plasma calcium, and is suppressed by elevated plasma calcium levels in the cow (Ramberg et al., 1967; Sherwood et al., 1966). Plasma phosphate level does not appear to be an important factor in the secretion of parathyroid hormone (Sherwood et al., 1968).

The mechanism by which plasma calcium concentration controls the secretion of parathyroid hormone appears to be proportional to the calcium concentration (Ramberg et al., 1967; Sherwood et al., 1968). In studying the role of parathyroid insufficiency in the etiology of parturient paresis, Mayer, Ramberg and Kronfeld (1969) observed hypocalcemia in parturient cows despite high levels of endogenous parathyroid hormone. Kronfeld (1969) suggests that the circulating hormone is inactive or that the target organs are unresponsive. Aurbach et al. (1969) suggests that the unresponsive nature of the target organs may be due to a lack or defect of parathyroid hormone sensitive adenyl cyclase in the organ. Work in humans (Estep et al., 1969) indicates that hypomagnesemia may be associated with the unresponsiveness to parathyroid hormone.

Evidence has been presented which suggests that cyclic adenosine-3',5'-monophosphate (cyclic AMP) mediates the action of parathyroid hormone (Rasmussen, Pechet and Fast, 1968; Wells and Lloyd, 1969). It was observed that perfusion of dibutyryl cyclic AMP into thyroparathyroidectomized rats caused changes which were the same as the effects of parathyroid hormone. Formation of cyclic AMP is catalyzed by adenyl cyclase (Aurbach and Houston, 1968). Parathyroid hormone causes stimulation of adenyl cyclase only in bone and kidney, although the enzyme is present in other tissues (Chase, Fedak and Aurbach, 1969). Though unexplored in the ruminant, it appears that in other species cyclic AMP is involved in the action of parathyroid hormone and that the physiological effects of parathyroid hormone involve the stimulation of adenyl cyclase in both bone and kidney. The exact mechanism of action of cyclic AMP is unknown.

It is well established that parathyroid hormone is involved in bone

resorption. Several theories have been proposed regarding the mechanism of action of parathyroid hormone and cyclic AMP in bone resorption (Aurbach et al., 1969; Chase et al., 1969; Martin et al., 1958; Rasmussen et al., 1967).

The major effect of parathyroid hormone on the kidney appears not to be promotion of calcium retention (Mayer, Ramberg, and Kronfeld, 1967), although it has been reported (Kleeman, Rockney and Maxwell, 1958). However, it is evident that parathyroid hormone causes an increase in urinary phosphorus by decreasing phosphorus reabsorption in the renal tubule (Mayer, Marshak and Kronfeld, 1966).

The effect of parathyroid hormone on calcium absorption from the gastrointestinal tract is unsettled. Rasmussen (1959) observed that parathyroid extract increased transport of calcium in isolated sacs of the small intestine of the rat. Cramer (1963) observed increased absorption of calcium from intestinal loops in dogs given parathyroid hormone. However, adult thyroparathyroidectomized cows have been observed to survive gestation, parturition and lactation without problems if adequate calcium was included in the diet (Mayer, Ramberg and Kronfeld, 1966). This would appear to minimize the role of parathyroid hormone in the absorption of calcium in the cow fed adequate dietary calcium.

Calcitonin

Since the discovery of calcitonin by Copp, Davidson and Cheney (1961), as sited by Copp (1969), the growth of knowledge concerning it has been phenomenal. Hirsch, Gauthier and Munson (1963) confirmed that the origin of calcitonin was the thyroid gland. They observed that acid

extracts of rat thyroid had a hypocalcemic and hypophosphatemic effect when injected into young rats. High calcium gland-perfusion studies in the pig also confirmed the thyroid origin of calcitonin (Care, 1965). The use of goats allowed Foster et al. (1964) to perfuse the superior parathyroid glands and the thyroid gland separately. A marked hypocalcemic effect was noted after perfusion of the thyroid, however, this was not observed after perfusion of the parathyroids.

Using immunofluorescent techniques, Bussolati and Pearse (1967) demonstrated the presence of calcitonin in the parafollicular 'C' cells of the thyroid gland of the dog and pig. The cells were referred to as 'C' cells because of their role in calcitonin production. Calcitonin was not present in the colloid-containing cells of the thyroid. Evidence that the parafollicular 'C' cells in mammalian thyroid glands are of ultimobranchial origin has been presented by Copp, Cockcroft and Kueh (1967) and Tauber (1967). They observed high concentrations of calcitonin in the ultimobranchial glands of chickens and turkeys with no calcitonin in the thyroid glands which contain no 'C' cells. In mammals, with the exception of the scaly anteater, the ultimobranchial gland becomes embedded in the thyroid gland (Pearse and Carvalheira, 1967). Depending upon specie, the parafollicular 'C' cells can be found either in the parathyroid and/or adjacent thyroid tissue (Care, Keynes and Duncan, 1966). Thus, calcitonin secretion should be associated with ultimobranchial origin rather than with thyroid origin alone.

Copp et al. (1962) observed that calcitonin secretion could be produced by raising the level of ionic calcium in the perfusing blood. In in vivo studies with pigs, Care (1969) found that the rate of calcitonin secretion was directly related to the level of plasma calcium. Similar

results have been observed in adult sheep (Copp, 1969) and in pigs and rabbits (Deftos, Lee and Potts, 1968; Lee, Deftos and Potts, 1969). Deftos et al. (1968) showed that the hormone was secreted continuously at normal concentrations of blood calcium in rabbits and pigs. They observed a rapid response to hypercalcemia with a calcitonin half-life in blood ranging from 5 to 15 minutes, which indicates a very rapid turnover rate. The normal thyroid gland appears to contain a reserve supply of calcitonin (Care, 1967).

The primary mode of action of calcitonin in causing hypocalcemia appears to be in its inhibition of bone resorption. O'Riordan and Aurbach (1968) injected calcitonin subcutaneously into rats 3 hours after an intravenous injection of radiocalcium. They observed an interruption in the rate of fall in specific activity of calcium in the blood. This indicated a reduced rate of entry into the blood which was interpreted to be due to decreased bone resorption. If calcitonin influences mineral accretion in bone, one would have expected the rate of radioactive calcium disappearance to have increased when calcitonin was injected. In work with chickens, Brown et al. (1969) concluded that calcitonin has no major role in bone growth. The release of calcium from bone cultures from mice (Aliapoulios, Goldhaber and Munson, 1966) and rats (Raisz et al., 1967) was inhibited by calcitonin. Martin, Robinson and MacIntyre (1966) noted that the administration of calcitonin reduced the excretion of hydroxyproline. This was attributed to reduced breakdown of bone collagen. Contrary to evidence that calcitonin does not influence bone growth, Foster et al. (1966) observed that rats treated with calcitonin exhibited reduced osteoclast count, increased trabecular bone and greater cortical bone density.

Thus, it appears that calcitonin exerts its hypocalcemic effect by inhibiting bone resorption. The hypocalcemic effect has been observed when the intestine was removed (Aliapoulios et al., 1966) and in nephrectomized animals (Hirsch, Voelkel and Munson, 1964). Calcium absorption in the rat duodenum was not altered by calcitonin in an in vivo perfusion study (Krawitt, 1967). Kenny and Heiskell (1965) observed no significant changes in calcium and phosphate of soft tissues after administration of calcitonin. The exclusion of the intestine, kidney and other soft tissues from influencing the hypocalcemic effect of calcitonin leads one to conclude that bone could be logical site of action.

The effects of calcitonin on the kidney appear to be quite variable. Clark and Kenny (1969) observed that injected porcine calcitonin had no direct effects on urinary excretion of calcium, phosphorus, magnesium or sodium by the dog kidney. In rats, calcitonin has been reported to have a phosphaturic effect (Kenny and Heiskell, 1965; Robinson, Martin and MacIntyre, 1966). Kenny and Heiskell (1965) observed no change in calcium excretion in rats administered porcine calcitonin.

Calcitonin action may be involved in phosphate metabolism. Recently, Talmage, Neuenschwander and Minkin (1969) demonstrated that endogenous calcitonin secretion could be stimulated by high plasma phosphate levels in the rat. In the dog, it has been observed that a hypophosphatemic response parallels a hypocalcemic response following injection of thyroid extracts (Chausmer, Mittleman and Wallach, 1966). These authors noted no consistent effect of calcitonin on plasma magnesium concentrations.

Vitamin D

Since about 1844, rickets has been recognized as a calcium deficiency disease. In 1922, the antirachitic factor in codliver oil was designated as vitamin D (Wright, 1969). Thus, the physiological relationship between vitamin D and calcium metabolism has been recognized for many years.

Vitamin D is often associated with normal calcification of bone. However, there is no evidence which indicates that vitamin D is directly involved in the calcification process (DeLuca, 1967). The physiological significance of vitamin D appears to involve its action in elevating plasma calcium and phosphate which in turn permits normal bone calcification (DeLuca, 1967). Elevation of plasma calcium and phosphate is implemented by the action of vitamin D in a calcium transport system in the intestine and by its action in the mobilization of constituents of deep bone. Parathyroid hormone is involved in the process of bone constituent mobilization which requires vitamin D (DeLuca, 1967).

One of the outstanding characteristics of vitamin D action is that there is a time lag between administration of the vitamin and the initiation of all of its recognized physiological responses (DeLuca, 1967; Norman et al., 1969). Norman (1966) observed that when vitamin D_3 was administered orally, intraperitoneally and intracardially in doses of 10, 100 or 20,000 IU, the lag in response of chick intestinal calcium transport was 15 to 25, 10 to 15 and 5 to 8 hours, respectively.

When vitamin D is administered orally to rats, it is absorbed primarily in the jejunum, with some if the ileal portion of the small intestine, in a process which requires bile (Norman and DeLuca, 1963; Schachter, Finkelstein and Kowarski, 1964). The absorption process

requires 4 to 6 hours (Zull, Czarnowska-Misztal and DeLuca, 1965). According to Schachter et al. (1964) the absorbed vitamin enters the lymphatic system, where a large portion is carried in the chylomicron fraction to the bloodstream. Transportation of the vitamin to various organs and tissues does not appear to be an important factor in the lag time. Norman et al. (1969) observed that an intracardial dose of 50 IU of 1,2 ^{3}H-vitamin D_3 to rachitic chicks resulted in a significant amount of radioactivity in the liver, skeleton, kidneys and intestinal mucosa within a short period of time.

Using silicic acid chromatographic profiles of tissue extracts of vitamin D-deficient rats given 10 IU of 1,2 ^{3}H-vitamin D_3 intravenously 24 hours before killing, Lund and DeLuca (1966) demonstrated that vitamin D_3 is not the principle form of the vitamin found in the tissues. Oral administration of this principle form or active metabolite to D-deficient rats resulted in a much more rapid intestinal calcium response than did an equivalent amount of vitamin D_3. Blunt, DeLuca and Schnoes (1968) identified the active metabolite of vitamin D_3 as being 25-hydroxycholecalciferol (25-HCC). Trummel et al. (1969) demonstrated that bone calcium mobilization in organ cultures was possible when 0.9 IU/ml of 25-HCC was added, however, 400 IU/ml of vitamin D_3 had no effect.

Recently, the active form of vitamin D_2 has been identified as 25-hydroxyergocalciferol (Suda et al., 1969). It appears that the liver is the major or only site for transformation of vitamin D_3 in the rat (Ponchon and DeLuca, 1969). Perfused liver and liver homogenates are both capable of transforming vitamin D_3 to 25-HCC in vitro (Horsting and DeLuca, 1969).

It has been shown that actinomycin D, an antibiotic that inhibits the DNA directed synthesis of RNA, when administered prior to vitamin D, can block the physiological response (Zull, Czarnowska-Misztal and DeLuca, 1966). DeLuca (1969) suggests that 25-HCC somehow causes DNA transcription into messenger RNA which inturn codes for one or more proteins which function in calcium transport.

It appears that a calcium-binding protein (CaBP) plus other proteins may be required for calcium transport. A CaBP which appears in the cells near the time of the increase in active transport of calcium, induced by vitamin D, has been isolated from the chicken duodenum, kidney and shell gland in the uterus of the chicken (Taylor and Wasserman, 1967; Wasserman, Corradino and Taylor, 1968; Wasserman and Taylor, 1966). It has also been found in intestinal homogenates from rats (Kallfelz, Taylor and Wasserman, 1967), dogs (Taylor, Wasserman and Jowsey, 1968) and monkeys (Wasserman et al., 1968). However, according to Harmeyer and DeLuca (1969) the appearance of the CaBP does not correlate well with calcium absorption. Recently, Martin, Melancon and DeLuca (1969) have detected a calcium dependent adenosine triphosphatase (ATPase) in the brush borders of the rat small intestine which is greatly increased after administration of vitamin D to rats.

DeLuca (1969) has proposed that the calcium dependent ATPase and the CaBP combine to form a system which when elucidated will explain the vitamin D mediated transport of calcium across the intestinal wall. This proposal tends to substantiate the two step mechanism explored by Schachter et al. (1966).

The calcium transport system of the intestine appears to be an active, cation oriented transport system in which phosphate is trans-

ferred secondarily to the calcium (Martin and DeLuca, 1969; Wasserman, Kallfelz and Comar, 1960). The transport mechanism in the duodenum of the rat appears to be quite specific for calcium ions in that strontium, barium and magnesium ions are not readily transported (Schachter, Dowdle and Schenker, 1960a). Recent in vivo studies with rats indicate that feeding magnesium ions may increase intestinal absorption of calcium and strontium (Clark, 1968). Sodium ions are required by the calcium transport system (DeLuca, 1967; Martin and DeLuca, 1969; Wasserman et al., 1960). Vitamin D is effective in stimulating calcium uptake by the mucosa from the lumen under sodium deficient conditions, however, it appears that sodium is required for the transfer of the calcium from the columnar epithelium to the serosa (Martin and DeLuca, 1969). It has been observed that calcium transfer is subject to competitive inhibition by ferrous ion (Manis and Schachter, 1962) and by potassium ion (Schachter, Dowdle and Schenker, 1960b).

In compiling information relative to the mechanisms involved in calcium homeostasis, it was observed that very little information has been accumulated by using the ruminant animal directly, with the exception of research concerned with parturient paresis in dairy cows. For this reason, the mechanisms reviewed must be interpreted with caution when applying them to the ruminant animal. Current evidence indicates that the mechanisms presented are operative in the ruminant animal.

Nutritional Aspects of the Metabolism of Certain Minerals

The subsequent portion of this review will be concerned with the nutritional aspects of calcium metabolism. Particular reference will be made to dietary calcium levels above those normally considered ade-

quate for the ruminant. The relationship of high dietary levels of calcium to the nutritional aspects of digestion and metabolism of calcium, phosphorus, magnesium, copper, zinc and potassium will be reviewed. An attempt has been made to restrict the following review to results that have been obtained with ruminant animals.

Mineral Availability

In the study of mineral metabolism, it is generally recognized that the level of a mineral element in a particular diet has little significance unless it is qualified by a factor indicating its availability to the specie involved. The term availability has been defined in several ways. Hill (1961) refers to the availability of a mineral as being analogous to the digestibility of an organic nutrient. According to Hill (1961), digestibility is an unsatisfactory term for several reasons. First, digestion of mineral salts or ions is impossible. Second, the availability of mineral elements is highly dependent upon feeding conditions and basic properties of the dietary constituents. Digestibilities of organic nutrients do change according to feeding conditions, however, these differences are small when compared to the differences which might occur with minerals. Care (1965) has defined availability as the fraction that is absorbed relative to the total amount of a mineral element presented to the absorptive area of the digestive tract.

The determination of true availability requires knowledge of endogenous mineral secretion and/or excretion. Though some controversy exists regarding their validity under certain conditions, the use of radiochemical procedures has greatly facilitated this process. Radioisotope procedures have been used to determine true availability of var-

ious dietary minerals in ruminants, particularly calcium (Hansard, Comar and Plumlee, 1954; Lueker and Lofgreen, 1961), phosphorus (Lueker and Lofgreen, 1961; Tillman, Brethour and Hansard, 1959; Young et al., 1966), magnesium (Care et al., 1967; Simesen et al., 1962) and zinc (Feaster et al., 1954; Miller and Cragle, 1965; Miller et al., 1968).

Calcium Availability

Using radioisotopes, Hansard, Crowder and Lyke (1957) studied the availability of calcium for cattle in 15 organic and inorganic sources of calcium. Though not statistically significant, availability from inorganic calcium sources tended to be higher than from alfalfa, lespedeza or orchard grass hays. Due to a limited number of experimental animals, no significant differences were detected among inorganic calcium sources. In comparing dolomitic limestone and calcium carbonate as calcium sources for yearling steers, Gerken and Fontenot (1967) found that calcium absorption and retention was lower for the dolomitic limestone supplemented ration.

Oxalate, a constituent of various plants, is known to lower the availability of calcium in the diets of nonruminants (Hill, 1961). However, soluble oxalate can be digested, i.e., broken down, by the ruminant, probably in the rumen, and this lowers the amount of oxalate available to combine with calcium (Talapatra, Ray and Sen, 1948).

Chapman et al. (1955) suggested that phytate may reduce the availability of calcium in swine diets. However, using sheep, Tillman and Brethour (1958) found no differences in the availabilities of calcium and phosphorus from calcium phytate and monocalcium phosphate. More recently, Dutton and Fontenot (1967) used magnesium oxide to supply

0.13 and 0.26% magnesium to yearling wethers. Phytic acid and mono- and disodium phosphate served as phosphorus sources at each level of magnesium. No differences in calcium absorption were observed.

In sheep, the rate of calcium absorption has been shown to increase with increasing calcium intake when the diet contains adequate phosphorus (Lueker and Lofgreen, 1961; Young et al., 1966). Calcium absorption is reduced by deficient levels of phosphorus in the diet (Young et al., 1966). The addition of 0.5% or more of zinc, as sulfate, to the diet of lambs resulted in a significant decrease in calcium absorption (Thompson, Hansard and Bell, 1959).

No relationship has been established regarding the influence of copper upon calcium availability. The only relationship between potassium and calcium absorption appears to be a hypothesis, discussed by Ward (1966), that high potassium levels reduce calcium absorption in ruminants. In vitro studies by Schachter et al. (1960b) indicate that calcium transfer may be subject to competitive inhibition by potassium ions.

The age of an animal may be related to the availability of calcium. Hansard et al. (1954) observed that availability values were greatest among young calves. The availability decreased rapidly to sexual maturity, then more slowly to maturity and again decreased in aged cattle. In comparing the availability of calcium from various sources, Hansard et al. (1957) observed that 5 to 7 month old calves exhibited greater absorption than mature steers. Work by Lengemann, Comar and Wasserman (1957) suggests that some of the observed differences could be accounted for by the type of diet fed the animals in the various age groups.

Phosphorus Availability

The availability of phosphorus to cattle and sheep appears to be greater, in general, than that of calcium (Conrad, Hansard and Hibbs, 1956; Kleiber et al., 1951; Shroder and Hansard, 1958; Thompson et al., 1959). Chapman et al., (1955) found that phytate phosphorus is relatively unavailable to pigs. However, sheep have the ability to utilize phytate phosphorus (Tillman and Brethour, 1958; Dutton and Fontenot, 1967). Lofgreen (1960) observed that significantly less phosphorus was absorbed from calcium phytate than that from dicalcium phosphate and bonemeal. In comparing various sources of phosphorus for lambs, Ammerman et al. (1957) observed that dicalcium phosphate and Curacao Island phosphate were superior to soft phosphate and defluorinated rock phosphate. Calcium metaphosphate and calcium pyrophosphate, substances which may be formed during thermal defluorination of rock phosphate are poorly utilized as sources of phosphorus for lambs.

Phosphorus status of the animal can have an effect on phosphorus availability. Young et al. (1966) observed that lambs fed a low phosphorus diet for 142 days exhibited enhanced phosphorus absorption when phosphorus intake was increased. However, the enhanced absorption persisted for only about 12 days after the increase in phosphorus intake.

The addition of aluminum sulfate to the diet of growing lambs has no appreciable effect on the availability of phosphorus (Thompson et al., 1959). A wide Ca:P ratio, up to 10.4:1 has no apparent effect on phosphorus absorption in sheep as long as phosphorus is adequate (Young et al., 1966). Similar effects were observed by Lueker and Lofgreen (1961) using ratios between 0.8:1 and 6:1. It was noted that the amount of phosphorus absorbed was directly related to the amount fed, however,

the availability was lowered when a diet having a wide Ca:P ratio contained a deficient level of phosphorus.

Dutton and Fontenot (1967) observed that magnesium levels of 0.13 and 0.26% had no effect upon phosphorus absorption in yearling wethers. Davis, Kidder and Becker (1953) reported that high levels of molybdenum in conjunction with low copper levels resulted in a decrease in phosphorus absorption in cattle. The effect of zinc or potassium on phosphorus absorption in ruminants has not been established.

Magnesium Availability

Active absorption of magnesium from the digestive tract against an electrochemical gradient has not been demonstrated, nor is there other evidence which indicates that magnesium is actively absorbed. It appears that magnesium has a different mechanism of absorption in the duodenum than calcium (Schachter et al., 1960a) and that it is not appreciably influenced by vitamin D (Smith, 1958).

The amount of magnesium absorbed in ruminants appears to increase as intake increases (Dutton and Fontenot, 1967; Smith, 1959), however, as this occurs the absorptive efficiency may decrease (Care and van't Klooster, 1965). The availability of magnesium is greater from magnesium oxide than from dolomitic limestone when fed to yearling steers (Gerken and Fontenot, 1967). In general, magnesium availability from hay or grain (approximately 17 to 38%) for ruminants appears to be lower than that of calcium or phosphorus (Care, 1965).

The magnesium status of the animal appears to effect the efficiency of magnesium absorption. McAleese, Bell and Forbes (1961) fed either a normal or magnesium-deficient diet for 4 weeks to lambs ranging from 3

to 5 months of age. It was observed that the deficient lambs absorbed a greater percentage of the magnesium fed.

Evidence indicates that calcium concentration may be related to magnesium availability in the calf and in vitro, however, the mechanism may involve other compounds such as ammonium and phosphate (Smith and McAllan, 1966; Smith and McAllan, 1967). It was observed that precipitation of magnesium with high levels of phosphate could occur if the pH was above 6.5. Using isolated loops of the intestine of sheep, Care and van't Klooster (1965) observed that the absorption of magnesium in the ileal loop decreased with increasing calcium concentration. Phosphorus supplied by phytate phosphorus or inorganic phosphorus at a level of 0.45% to yearling wethers has no effect on magnesium absorption (Dutton and Fontenot, 1967).

The effects of copper and zinc upon magnesium absorption in the ruminant have not been established. Much work has been devoted to studying the relationship of potassium to magnesium, especially in relation to ruminant hypomagnesemia. However, a direct relationship between potassium and magnesium absorption has not been established.

Copper Availability

The effects of calcium, phosphorus, magnesium, copper, zinc and potassium upon the absorption of copper have not been established in ruminants.

Zinc Availability

Little work has been conducted to determine the effects of calcium, phosphorus, magnesium, copper, zinc and potassium upon the absorption

of zinc in ruminants. It has been demonstrated in pigs (Oberleas, Muhrer and O'Dell, 1962) and rats (Oberleas, Muhrer and O'Dell, 1966) that when the diet contained phytate, excess calcium accentuated zinc deficiency symptoms, however, this did not occur when phytate was not present. Hydrolysis of phytate can occur in the rumen (Raun, Cheng and Burroughs, 1956) which would probably destroy its zinc binding capacity.

In dairy cows, Miller and Cragle (1965) observed that approximately 35% of the zinc fed was absorbed from the abomasum. At pH levels normally found in the abomasum, very little if any calcium or zinc would complex with phytate. Therefore, high levels of calcium in a ruminant diet, containing adequate zinc, would not be expected to precipitate a zinc deficiency by decreasing the availability of zinc. Though absorption data were not obtained, Mills and Dalgarno (1967) observed zinc deficiency lesions when lambs were fed high levels of calcium.

Potassium Availability

Ruminants normally consume amounts of potassium which exceed their dietary requirement. A deficiency of potassium would rarely, if ever, occur on any natural diet consumed by ruminants (Ward, 1966). For these reasons, little or no work has been conducted on the effects of calcium, phosphorus, magnesium, copper, zinc and potassium upon the absorption of potassium in ruminants.

Site(s) of Absorption

Calcium

When directly adding ^{45}Ca to the abomasum of young calves maintained on a hay and grain diet, Chandler *et al.* (1969) observed that an

average of 20.8% of an administered dose of ^{45}Ca was absorbed from the abomasum within 25 minutes. According to Storry (1961a), there is essentially no bound calcium in the abomasum, where the pH is normally 2 to 3. Thus, the calcium would be in its best possible state for absorption. The proportion of bound calcium in the small intestine appeared to be directly related to the pH of the digesta. Storry (1961b) noted in an in vitro experiment that as the pH of digesta increased calcium and magnesium ions were preferentially bound to suspended material of the digesta instead of precipitating as calcium phosphate or magnesium ammonium phosphate. However, in the absence of digesta, the precipitation of phosphates occurred above pH 6.0.

Hogan and Phillipson (1960) demonstrated that the pH of digesta in the sheep's duodenum varied from 1.6 to 3.4. Storry (1961a) noted that the pH of the digesta in the proximal section (274 cm) of the sheep's small intestine ranged from 5.2 to 6.2. The high pH values were attributed to the length of the section. Phillipson and Storry (1965) reported pH values from 2.5 to 4.5 in the duodenum and jejunum of sheep. From these results it appears that the pH of the digesta in the proximal portion of the small intestine of sheep is relatively low. The low pH should impede the binding of calcium.

Phillipson and Storry (1965) observed no significant disappearance of calcium from the rumen. It was observed that a considerable amount of calcium absorption occurred in the duodenum, however, it did not surpass the endogenous calcium secreted into the duodenum by the liver, pancreas and Brunner's glands. Storry (1961c) found that bile and pancreatic juice contain considerable quantities of calcium and magnesium.

Phosphorus

The site of phosphorus absorption in ruminants is not well defined. However, work in nonruminants indicates that phosphate is transported secondarily in the active calcium transport system (Martin and DeLuca, 1969; Wasserman, Kallfelz and Comar, 1960) which is known to be active in the proximal small intestine. It appears that phosphorus may be absorbed from the proximal small intestine in ruminants (Chandler and Cragle, 1962; Wright, 1955).

Magnesium

Storry (1961a,b) observed that the proportion of bound magnesium was lowest in the abomasum and duodenum of sheep. The major site of magnesium absorption appears to be distal to the duodenum in the sheep (Care and van't Klooster, 1965; Field, 1961; Scott, 1965). Phillipson and Storry (1965) observed significant absorption from the rumen and all sections of the small intestine, however, these results were erratic.

Copper

Little is known about the mechanism responsible for copper absorption or the site(s) of absorption in the ruminant.

Zinc

Miller and Cragle (1965) observed that approximately 35% of the daily administered ^{65}Zn was absorbed from the abomasum of dairy cattle. Secretion of zinc into the duodenum far surpassed that absorbed. Net absorption occurred in the jejunum and ileum with little excretion or absorption occurring beyond the cecum. In addition, it was observed

that the magnitude of zinc absorption from the abomasum and resecretion into the duodenum did not differ in milk-fed calves and older animals fed hay and grain. However, the overall zinc balance was 55% in baby calves, 20% in month old calves and 12% in cows.

Potassium

It appears that potassium enters the blood by a diffusion process. Much potassium is absorbed from the rumen (Parthasarthy and Phillipson, 1953; Perry, Cragle and Miller, 1967) and from the omasum (Oyaert and Bouckaert, 1961).

Mineral Balance

Mineral balance studies have certain limitations relative to the interpretation of mineral metabolism in ruminants (Duncan, 1958; Hill, 1961; Thompson, 1965). However, the simplicity of the method has promoted its use. Thompson (1965) suggests that it can be of value in estimating the requirements of a growing animal, however, it is of little value in mature animals. Interpretation of the results obtained from balance studies must be made with caution.

In an early study, Lindsey (1931), as cited by Duncan (1958), imposed a high and low calcium level upon two groups of dairy heifers for the first 3 years of their lives during which short balance trials were conducted. It was observed that the amount of calcium retained, as a percent of intake, was almost the same for the two groups. Thus, the animals on the high calcium level retained more calcium. Animals on the high calcium level retained more phosphorus than the heifers on the low calcium level. Live weight growth was nearly identical for the groups.

In a similar experiment, Archibald and Bennett (1935), as cited by Duncan (1958), used high and low levels of phosphorus. The amount of calcium retained by the two groups of dairy heifers was nearly the same, however, the animals on the low level of phosphorus retained a greater percent of the phosphorus ingested.

In more recent work Lueker and Lofgreen (1961) observed that the amount of calcium or phosphorus absorbed was directly related to the amount fed, however, the metabolic fecal loss of calcium was independent of the amount of calcium or phosphorus absorbed. In contrast, the amount of metabolic fecal phosphorus excreted increased as the phosphorus absorption increased and decreased as the amount of calcium absorbed increased. Metabolic fecal phosphorus excretion was lowest with a ration containing 6.0:1 calcium to phosphorus ratio. Visek et al. (1953) noted that metabolic fecal calcium loss in cattle was independent of dietary intake. Using nonpregnant dairy cows, Manston (1967) found that as the dietary intake of calcium increased, the percentage of dietary calcium absorbed remained nearly constant. Although the endogenous calcium loss increased slightly with increased absorption, the calcium balance did increase as the amount absorbed increased. Preston and Pfander (1964) observed that the phosphorus balance of growing lambs increased as dietary phosphorus increased.

Dutton and Fontenot (1967) found that as magnesium absorption increased in yearling wethers, fecal magnesium and magnesium balance increased. When potassium was added to the diet of 2 year old wethers, Suttle and Field (1967) observed no change in magnesium and phosphorus retention. It did appear to alter the partition of ingested magnesium and phosphorus between the urine and feces.

The influence of high levels of dietary calcium upon the balance of magnesium, copper, zinc and potassium has received little or no attention in ruminants.

General Conclusion

This review of literature indicates the paucity of information concerning the metabolism of calcium, phosphorus, magnesium, copper, zinc and potassium in lambs as related to dietary calcium level. Therefore, this study was undertaken to evaluate the nutritional aspects of the effects of the dietary level of calcium, in particular those levels above the requirements of ruminants, upon the metabolism of these mineral elements.

CHAPTER III

MATERIALS AND METHODS

Introduction

Four levels of dietary calcium were evaluated in a completely randomized experimental design with five lambs per treatment. Purified diets containing 0.58, 1.60, 2.79 and 4.46% calcium were fed during a 231-day experimental period consisting of two phases: an 84-day balance phase followed by a 147-day growth phase. Appropriate samples and measurements were obtained at 21-day intervals throughout the study. Upon termination of the growth phase, three lambs were selected at random from each treatment group to study the effect of dietary calcium level upon alimentary tract pH. Carcasses from the lambs involved in the pH study were measured to evaluate skeletal development and various tissue samples were obtained.

Animals, Equipment and Diets

Twenty wether lambs (Hampshire x Western) approximately 3 months of age and weighing about 17 kg were placed at random in metabolism pens (140cm x 84cm). These pens were equipped with slatted floors and facilities for separation and collection of urine and feces. Movement within pens was unrestricted. To avoid mineral contamination, all facilities were constructed of wood or other organic materials. The

lambs were treated with thibenzole[1] as an antihelminth.

During a 21-day preliminary adjustment period the lambs were fed a purified diet designated as preliminary in Table I. Four purified diets were formulated with varying levels of calcium. Starch, dextrose and cellulose were replaced with calcium carbonate as the dietary level of calcium was increased. Based upon chemical analyses, the four experimental diets A, B, C and D contained 0.58, 1.60, 2.79 and 4.46% calcium, respectively, with calcium to phosphorus ratios of 1.54:1, 4.53:1, 7.84:1 and 12.52:1. All nutrient levels were above those currently recommended by the National Research Council (1964). Diets were mixed in batches of 91 kg from which representative samples were secured for subsequent chemical analyses of mineral content. Two samples from each batch were analyzed for calcium, inorganic phosphorus, magnesium, copper and zinc. The calculated levels of certain elements (based upon physical composition of the diets) and the levels of certain elements as determined by chemical analyses (averaged over samples and batches) are shown in Table II. To avoid mineral contamination from water, animals consumed distilled water throughout the 231-day study.

Balance Study

The initial phase of this experiment consisted of an 84-day balance study. The four treatments (diets) were randomly assigned to the lambs following a 21-day preliminary period. Thus, a completely randomized design was employed with five lambs per treatment. After 60 days on experiment, a peritoneal infection caused the death of one

[1]Merck and Co., Inc., Rahway, New Jersey.

TABLE I

COMPOSITION OF THE PURIFIED DIETS

Item	Diets				
	Prelim.	A	B	C	D
Starch, %	34.3585	34.3545	33.4835	32.6124	31.7414
Dextrose, %	24.3583	24.3089	23.6926	23.0763	22.4600
Cellulose, %	29.9886	30.0005	29.2399	28.4793	27.7186
Urea,[a] %	4.2000	4.2000	4.2000	4.2000	4.2000
Corn oil,[b] %	1.0000	1.0000	1.0000	1.0000	1.0000
Polyethylene resin,[c] %	1.0000	1.0000	1.0000	1.0000	1.0000
Choline chloride, %	0.1102	0.1102	0.1102	0.1102	0.1102
Vitamin A and D,[d] %	0.0110	0.0110	0.0110	0.0110	0.0110
Minerals, %	4.9042	5.0149	7.2628	9.5108	11.7588
Mineral composition, gm/100 kg diet					
$CaCO_3$			2247.9563	4495.9126	6743.8689
K_2CO_3	2216.9072	1953.6157	1953.6157	1953.6157	1953.6157
KCl		275.3917	275.3917	275.3917	275.3917
$CaHPO_4$	1325.2051				
$CaHPO_4 \cdot 2H_2O$		1288.2335	1288.2335	1288.2335	1288.2335
$NaH_2PO_4 \cdot H_2O$		303.6295	303.6295	303.6295	303.6295
$MgSO_4$	120.1725	120.1514	120.1514	120.1514	120.1514
$MgCO_3\ Mg(OH)_2 \cdot 3H_2O$	266.8050	266.7582	266.7582	266.7582	266.7582
Na_2SO_4	250.0470	250.0032	250.0032	250.0032	250.0032
$FeSO_4$	42.5565				
$FeSO_4 \cdot 7H_2O$		77.8661	77.8661	77.8661	77.8661
$ZnSO_4 \cdot 7H_2O$	26.3791	26.3766	26.3766	26.3766	26.3766
NaCl	625.1175	410.5081	410.5081	410.5081	410.5081
$MnSO_4 \cdot H_2O$	15.3865	15.3858	15.3858	15.3858	15.3858
$Na_2B_4O_7$	12.5685				
$Na_2B_4O_7 \cdot 10H_2O$		23.8125	23.8125	23.8125	23.8125
$CuCO_3$	1.9699	2.0322	2.0322	2.0322	2.0322
CaF_2	0.2000				
KI	0.0684	0.0681	0.0681	0.0681	0.0681
$CoCl_2 \cdot 6H_2O$	0.0450	0.0443	0.0443	0.0443	0.0443
$KF \cdot 2H_2O$		0.4806	0.4806	0.4806	0.4806
$NaMoO_4 \cdot 2H_2O$	0.5001	0.4993	0.4993	0.4993	0.4993
Na_2SeO_4	0.2491	0.0240	0.0240	0.0240	0.0240
$Cr(SO_4)_3 \cdot nH_2O$	0.0399	0.0414	0.0414	0.0414	0.0414

[a]Crystalline urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

[b]Mazola. 1,2 dihydroxy-6-ethoxy, 2,2,4,trimethyl quinoline (ethoxyquin) added to provide 0.0125% in total ration.

[c]Microthene. U.S. Industrial Chemicals Co., New York, N.Y.

[d]Diets contained 4,400 IU vitamin A and 550 IU vitamin D_3 per kg. NOPCO Chemical Co., Harrison, New Jersey.

TABLE II

MINERAL ELEMENT COMPOSITION OF THE PURIFIED DIETS

Item	Diets				
	Prelim.	A	B	C	D
Calcium,[a] %	0.7561	0.5785	1.6005	2.7883	4.4647
Phosphorus,[a] %	0.3567	0.3757	0.3533	0.3558	0.3565
Magnesium,[a] %	0.1042	0.1024	0.1027	0.1100	0.1141
Copper,[a] ppm	14.1420	13.1480	11.3920	13.7340	14.8130
Zinc,[a] ppm	56.5000	53.9680	56.8480	65.4900	50.5340
Other minerals[b]					

[a]Determined by chemical analysis.

[b]All diets were prepared to provide: K, 1.25%; S, 0.10%; Na, 0.33%; Cl, 0.38%; Fe, 156.41 ppm; Mn, 50.01 ppm; B, 27.02 ppm; I, 0.52 ppm; Co, 0.11 ppm; F, 0.97 ppm; Mo, 1.98 ppm; Se, 0.10 ppm; Cr, 0.11 ppm.

lamb in treatment A. Staff veterinarians diagnosed the infection as being unrelated to the treatment imposed. Another lamb in treatment A was removed from the experiment due to an injury incurred during shearing. Consequently, all results were computed on the basis that treatments A, B, C and D contained 3, 5, 5 and 5 animals, respectively. The animals were fed once daily to maximum feed consumption (the amount fed daily varied according to individual consumption patterns).

Urine was collected daily and a sample equivalent to one percent of the volume was taken and composited on a 21-day basis for subsequent analysis. All feces were collected, dried at approximately 50°C and composited on a 21-day basis. Dried feces were ground in a Wiley Mill[2] through a 2 mm screen. Following termination of the balance phase, 21-day composite quantities of dried, ground feces were thoroughly mixed and condensed with sample dividers. Approximately 500 gm samples (representing excreta from each lamb for 21 days) were obtained for subsequent analysis.

A wool sample was obtained initially and at 21-day intervals from the neck region (approximately a 38 cm x 25 cm area) of each lamb.

Blood samples were collected initially and at 21-day intervals by jugular puncture. Approximately 8 ml of heparinized (0.2 mg heparin per 8 ml of blood) blood and 25 ml of unheparinized blood were collected in polyethylene centrifuge tubes. Immediately following collection, the blood samples were placed in a cooler.

Growth Study

Immediately following the balance study, a 147-day growth study

[2]Arthur H. Thomas Co., Philadelphia, Penn.

was conducted using the same animals and treatments. All samples and measurements were obtained as they were during the balance phase with the exception of urine and feces collection. The growth study was conducted in order to observe changes which might occur if the lambs were allowed to approach market weight on the experimental diets.

Alimentary Tract pH

Preparation and Anesthesia

Upon termination of the growth phase, three lambs were selected at random from each treatment group and used to study the effect of dietary calcium level upon alimentary tract pH. Dietary calcium levels were identical to those used during the balance and growth studies. In order to standardize the digestive state of the lambs selected, feed was removed 24 hours prior to surgery. Five hours before surgery the animals were fed, and the amount of feed consumed during this period was recorded.

In preparing the animals for surgery, an area 15 cm wide over the median raphe and 10 cm wide over each jugular vein was closely clipped with an electric wool clipper. Each animal was weighed and the dosage of sodium pentobarbital[3] was calculated on the basis of 20 mg per kg of live weight. One half of the calculated dose was given rapidly via the right jugular vein. With the animal in lateral recumbency, sufficient additional anesthetic was given to place the animal in the second plane of stage three of surgical anesthesia (Wright and Hall, 1961). During the surgical procedure, anesthetic was intravenously

[3]Diabutal. Diamond Labs., Inc., Des Moines, Iowa.

administered as necessary to maintain anesthesia.

Surgical Procedure

An electrosurgical unit[4] was used for incisions and hemostasis. The abdomen was incised along the median raphe from the xiphiod cartilage to the pubis. The pylorus was exteriorized and selected segments of the small intestine were isolated using a rubber covered Mayo-Robson[5] forceps. Beginning at the pylorus, the following sections of the small intestine were isolated and sampled: four 30.5 cm sections, five 61 cm sections, subsequent samples were taken from 122 cm sections (the number of 122 cm sections which were sampled varied slightly according to variations in length of the small intestine), and the final sample was recovered near the ileo-cecal valve. Additional samples for pH determination were obtained from the right anterior ventral sac of the rumen, the fundic region of the abomasum (lateral to the greater curvature), the point of the blind sac of the cecum, at the point of the flexure centralis of the spiral colon and the lower colon (10 cm from the anal sphincter). Where possible, intestinal contents were removed by aspiration with a 20 guage x 3.81 cm hypodermic needle and syringe. In other cases, a bloodless incision 1 to 2 cm in length was made in the antimesenteric portion of each segment with the electrosurgical unit. The intestinal contents were then gently milked from the segment. Contamination of the ingesta by blood was prevented by the bloodless incision. Intestinal

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[4]Wappler Tube-Gap Surgical Unit. American Cystoscope Malcero, Inc., New York, N. Y.

[5]Miltex Surgical Instruments, New York, N. Y.

incisions were closed with Michel[6] skin clips. Following the entire operation which required approximately 90 minutes per lamb, the abdominal incisions were sutured. The animals were allowed to regain consciousness, however, before excessive movement could occur, each animal was killed by exsanguination. The carcasses were measured as described in the Data Obtained section and various tissue samples were obtained for subsequent mineral analysis.

Measurement of pH

Each sample from the alimentary tract was collected in a 95 cm x 22 cm petri dish. The pH was determined immediately with a pH meter[7] equipped with a glass, reference combination electrode capable of measuring the pH of small quantities (approximately 0.1 ml). Prior to pH determination, nonliquid samples were converted to slurries by adding glass distilled water and thoroughly mixing.

Data Obtained and Methods of Analysis

Sixteen hour shrunk weights (without feed and water) were obtained initially and at 21-day intervals during the balance and growth studies. Individual feed and water consumption were recorded. With the exception of urine and feces collection, all measurements and samples obtained were exactly the same during both studies. The amount of fecal material excreted by each animal was recorded during the balance phase.

Heparinized blood samples were used in determining hematocrit

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[6]Clay Adams, Inc., New York, N. Y.

[7]Corning Model 10. Corning Glass Works, Corning, N. Y.

(percent packed cell volume) and hemoglobin levels. Hematocrit was determined in duplicate according to the method described by McGovern, Jones and Steinberg (1955). Blood samples were centrifuged for 5 minutes at 10,000 rpm in an International Micro-Capillary Centrifuge[8]. Hemoglobin was determined in duplicate by using Hycel cyanmethemoglobin reagent[9]. Spectrophotometric reading of the resultant hemoglobin solutions was conducted on a Gilford Model 240[10] spectrophotometer at a wave length of 540 mμ.

Serum was separated by centrifuging[11] whole, clotted blood at a relative centrifugal force of 3000x gravity for 15 minutes at 5°C. The serum was removed by decantation, placed in sterile, stoppered plastic tubes and stored for one week at 4°C. At this time, serum alkaline phosphatase activity was determined in duplicate by using the Sigma 104 procedure[12]. The amount of p-nitrophenol liberated was determined by a Gilford Model 240[13] spectrophotometer. Immediately following determination of serum alkaline phosphatase, the serum samples were stored at -17°C.

Serum calcium, magnesium, copper, zinc and potassium were determined by atomic absorption spectrophotometry using a Perkin-

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[8] International Equipment Co., Needham Hts., Mass.

[9] Hycel, Inc., Houston, Texas

[10] Gilford Instrument Labs., Oberlin, Ohio

[11] International High-Speed Refrigerated Centrifuge Model HR-1. International Equipment Co., Needham Hts., Mass.

[12] Sigma Chemical Co., St. Louis, Mo.

[13] See footnote 10.

Elmer Model 303[14] spectrophotometer equipped with a digital concentration readout[15]. The methods used were as described by the manufacturer. Inorganic phosphorus was determined according to the procedure outlined by Fiske and Subbarow (1925), using a Baush and Lomb Spectronic 20[16]. In order to measure variation due to technique and instrumentation, duplicate analyses were conducted on each sample.

Urine calcium, phosphorus, magnesium, copper and zinc were determined by the methods outlined for serum.

Diets were mixed in batches of 91 kg from which representative samples (approximately 1 kg) were obtained and stored in plastic bags until analyses could be conducted. Duplicate 2 gm samples of air dry feed from each batch were ashed in a muffle furnace[17] for 8 hours at 575 to 600°C (this temperature was attained gradually). The ash was dissolved in a 1:3 HCl (one part concentrated HCl to three parts of glass distilled water), transferred to a 25 ml volumetric flask and diluted to volume with 1:3 HCl. Calcium, phosphorus, magnesium, copper and zinc were determined as outlined for serum.

Wool samples were dried for 10 to 12 hours at 100°C. The dried samples were extracted for 12 hours with diethyl ether on Goldfisch Extractors[18]. To insure complete extraction of ether soluble material,

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[14]Perkin-Elmer Corp., Norwalk, Conn.

[15]Model DCR 1. Perkin-Elmer Corp., Norwalk, Conn.

[16]Baush and Lomb, Rochester, N. Y.

[17]Hevi-Duty Electric Multiple Unit Furnace. Hevi-Duty Heating Equipment Co., Watertown, Wis.

[18]Laboratory Construction Co., Kansas City, Mo.

the samples were extracted with fresh ether for another 12 hour period. The samples were then carefully washed (20 to 50 washings) in glass distilled water until they appeared clean. The clean wool samples were then dried for 16 hours at 100°C and their dry weights recorded. Prior to ashing, each wool sample was charred with a Fisher burner. The samples were then ashed and dissolved in acid as described for feed samples. Ash weight was recorded. Duplicate determinations of calcium, phosphorus, magnesium, copper, zinc and potassium were conducted as described for serum.

Immediately following completion of the alimentary tract pH determinations, each animal was sacrificed. Upon slaughter the following measurements were recorded: heart, liver, combined kidney and hot carcass weights. Following a 48 hour chill at 4°C the following measurements were obtained: carcass length, length of leg and depth of body. Carcass length was determined by measuring the distance from the anterior edge of the first rib, adjacent to the vertebrae, to the anterior edge of the aitchbone. Length of leg was determined by measuring the distance from the anterior edge of the aitchbone to the furthest extremity of the leg. Depth of body was determined by measuring the distance from the ventral edge of the spinal canal at the fifth thoracic vertebra to the ventral edge of the sternum, on a line perpendicular to the length of the carcass.

A lean sample from each lamb was obtained from a mixture of the *longissimus dorsi* muscle (only that portion posterior to the 12th rib), the *semitendinosus* and *semimembranosus* muscles. A fat sample was taken from a mixture of the fat surrounding both kidneys. The femur and tibia (right and left) were carefully wrapped and stored at -17°C

for subsequent measurement. Lean and fat samples were placed in polypropylene bottles and stored at -17°C until chemical analyses could be conducted.

The femur and tibia (right and left) were dissected free of muscles, ligaments and periosteum, weighed and the length was measured. Specific gravity of each bone was obtained by hydrostatic weighing. The length of the femur was determined by measuring the distance from the trochanter major to the lateral condyle. The length of the tibia was measured from the medial condyle to the distial epiphysis. The cross sectional area of the cortex and medullary canal of each bone were determined by bisecting the bones, tracing the cross section on acetate paper and subsequently measuring the area of the cortex and medullary canal with a planimeter.

Prior to sampling, representative quantities of lean were blended into a homogenous paste in an omnimixer[19]. Duplicate 5 gm samples were dried for 48 hours at 100°C. Prior to ashing, the samples were charred with a Fisher burner. The samples were ashed and dissolved in acid as described for feed. With the following exceptions, the same procedure was used in preparing the kidneys for analysis. Duplicate 20 gm samples were dried for 72 hours prior to charring. Duplicate 15 gm quantities of fat were handled in the same manner as the kidneys. During the charring process, lean, kidney and fat samples were allowed to ignite and burn very slowly. Duplicate determinations of calcium, phosphorus, magnesium, copper, zinc and potassium content of lean, fat and kidney samples were conducted as previously indicated for feed and serum.

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[19]Sorvall Omni-Mixer. Ivan Sorvall Inc., Newton, Conn.

Analyses of variance were calculated according to the methods outlined by Steel and Torrie (1960). The mean square for animals within treatment was used for testing differences among treatments. The sources of variation and appropriate degrees of freedom for each type of analysis of variance conducted in this experiment are presented in Appendix Table IV. Response criteria analyzed by each type of analysis of variance are indicated in Appendix Table IV.

CHAPTER IV

RESULTS AND DISCUSSION

Animal Performance

Lamb weight and lamb weight change are portrayed graphically in Figures 1 and 2, respectively. Though no statistically significant differences existed, the greatest performance was achieved by those lambs which received 1.60% dietary calcium followed by those which received 0.58, 2.79 and 4.46%, respectively. Average daily gains during the balance and growth phases followed a very similar pattern (Appendix Table VII).

These results are in agreement with those of Wise et al. (1963) and Dowe et al. (1957). In their experiments, calcium to phosphorus ratios of 1:1 to 7:1 with adequate phosphorus did not cause significant differences in calf growth. Ratios above these levels resulted in a depression of growth, which tends to agree with the trends observed in this experiment. Though small numbers of calves were utilized, Swenson, Underbjerg and Goetsch (1956) and Colovos, Keener and Davis (1958) observed similar results.

The average daily feed consumption (Figure 3 and Appendix Table VIII) appeared to plateau for all treatments after the lambs had been on experiment for 18 weeks. This plateau could be due to a seasonal effect. No statistically significant treatment differences existed in feed consumption, although, average daily consumption for treatments B, A, C and

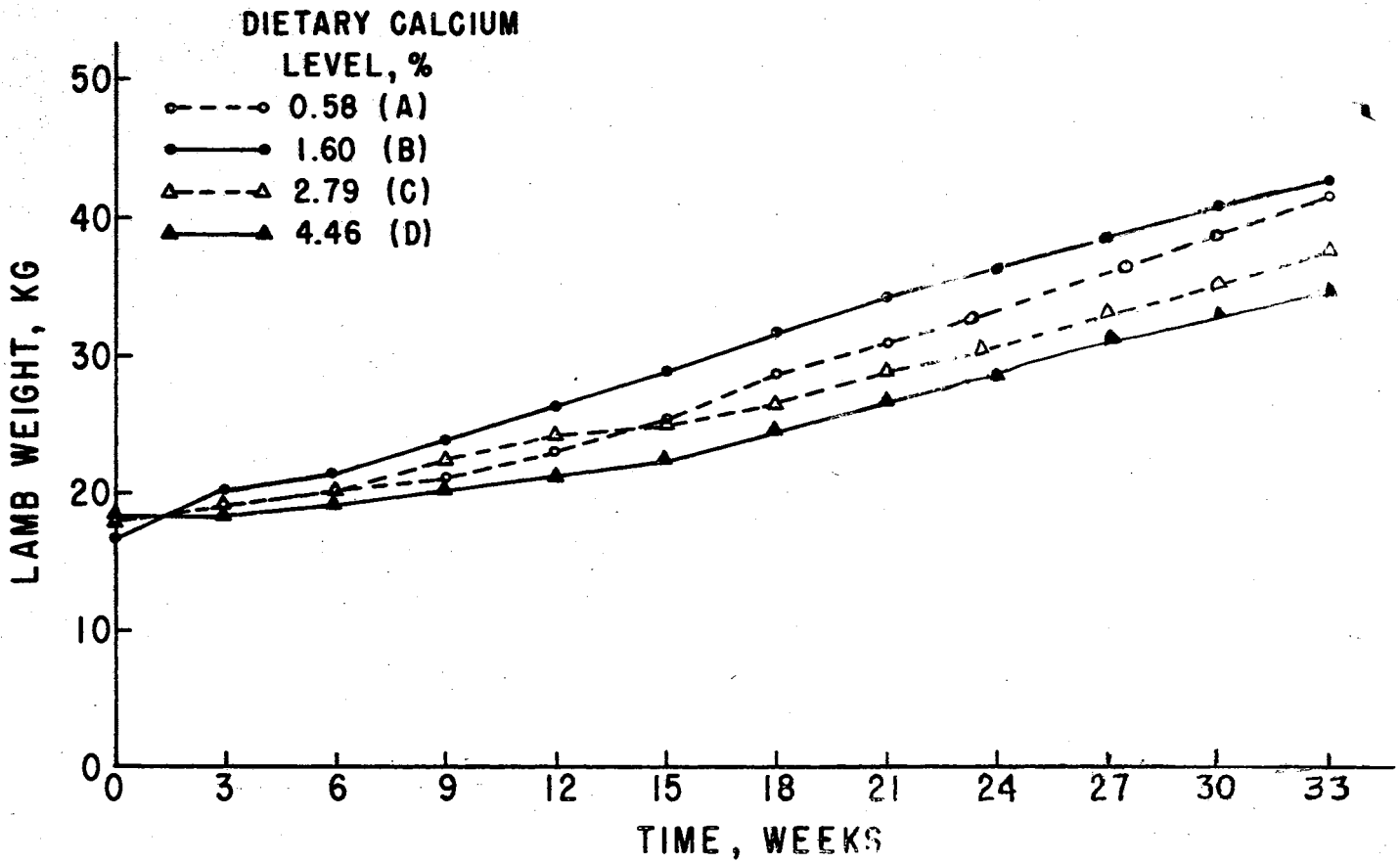

Figure 1. Absolute Weights of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table V.

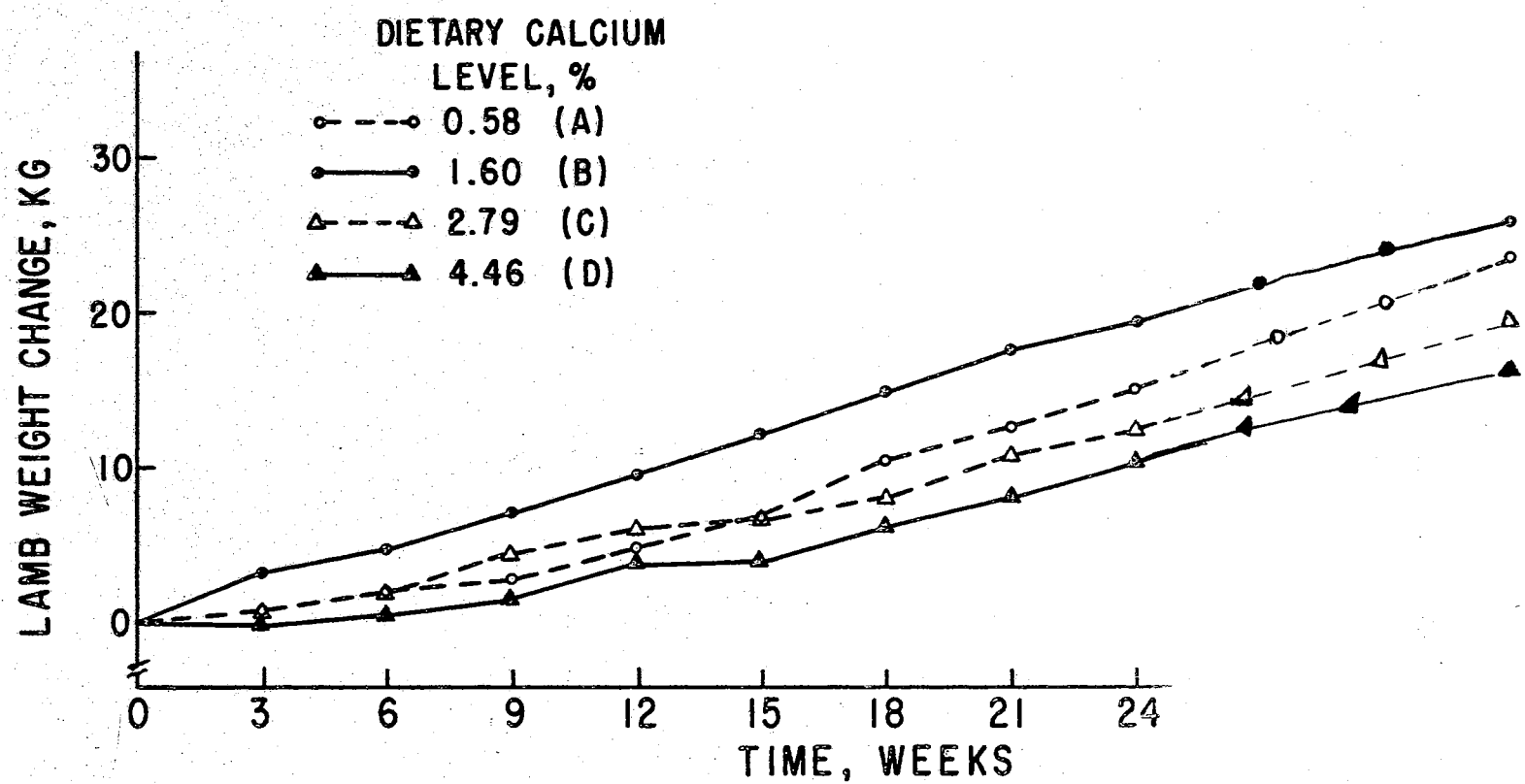

Figure 2. Lamb Weight Change of the Lambs Fed the Various Levels of Calcium, i.e., the Difference Between Initial Weight and the Weight at the Given Times. The Level of Significance Among the Overall Treatment Means was 0.296. These Data are Presented Numerically in Appendix Table VI.

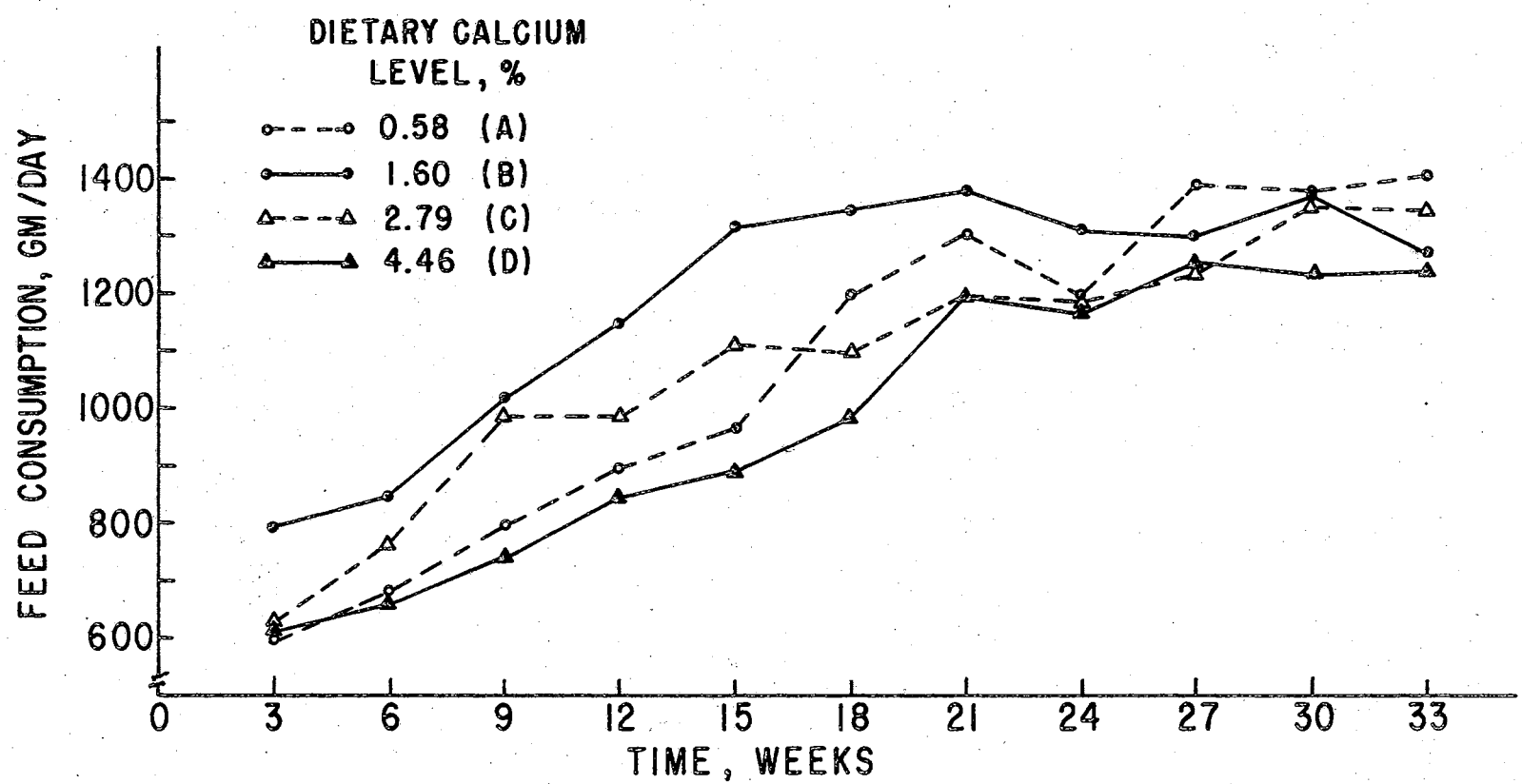

Figure 3. Average Daily Feed Consumption of the Lambs Fed the Various Levels of Calcium. Each Point Represents the Mean Daily Consumption for the Preceding 21-day Period. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table VIII.

D were 1192, 1073, 1073 and 983 gm per day, respectively. This trend in feed consumption is similar to that observed for lamb weight gain.

Using natural diets, Wise et al. (1963) and Dowe et al. (1957) observed no significant differences in feed intake when high levels of calcium, and diets with wide calcium to phosphorus ratios were fed to growing calves. The palatibility of the diets containing the higher levels of calcium may have influenced the feed consumption trends in the present experiment (Figure 3). Colovos et al. (1958) observed that high dietary levels of limestone depressed the digestibilities of both protein and energy for dairy heifers.

No significant treatment differences occurred in water consumption (Figure 4 and Appendix Table IX). The pattern observed appears to reflect changes in lamb size and seasonal effects.

Serum Alkaline Phosphatase Activity

The serum alkaline phosphatase activity observed in this experiment is illustrated in Figure 5. After adjusting for initial differences, there were no treatment differences and there appeared to be little or no treatment change as the lambs became older.

It appears that the role of bone alkaline phosphatase activity in bone growth has not been clarified. A decrease in bone alkaline phosphatase activity has been shown to coincide with a decrease in bone growth in rats (Wergedal, 1969a). In contrast, Wergedal (1969b) observed that rats fed vitamin D deficient diets exhibited elevated bone alkaline phosphatase activity. Alcock and Shils (1969) found no direct relationship between alkaline phosphatase activity of rat cartilage and the calcification process.

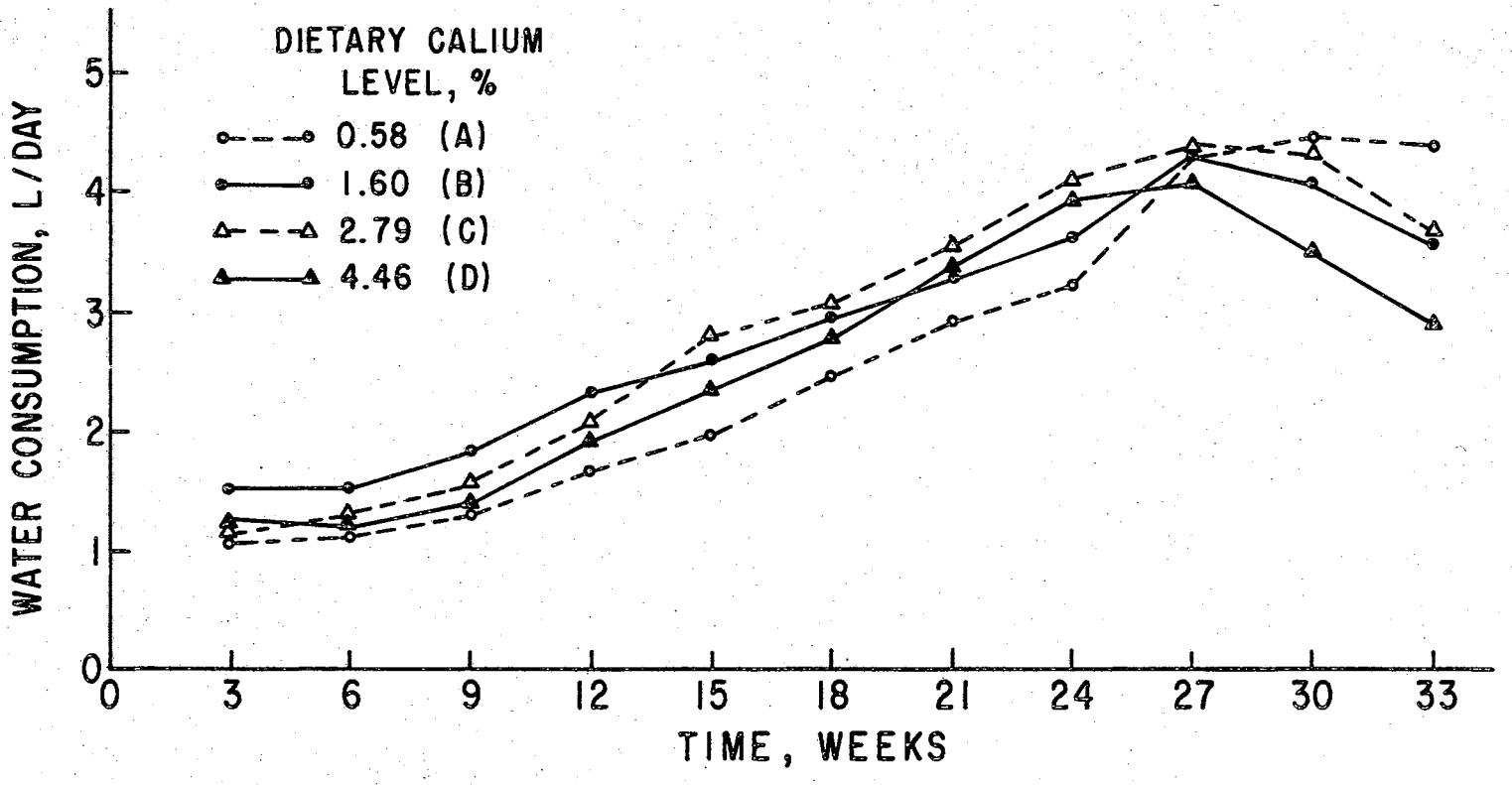

Figure 4. Average Daily Water Consumption of the Lambs Fed the Various Levels of Calcium. Each Point Represents the Mean Daily Consumption for the Preceding 21-day Period. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table IX.

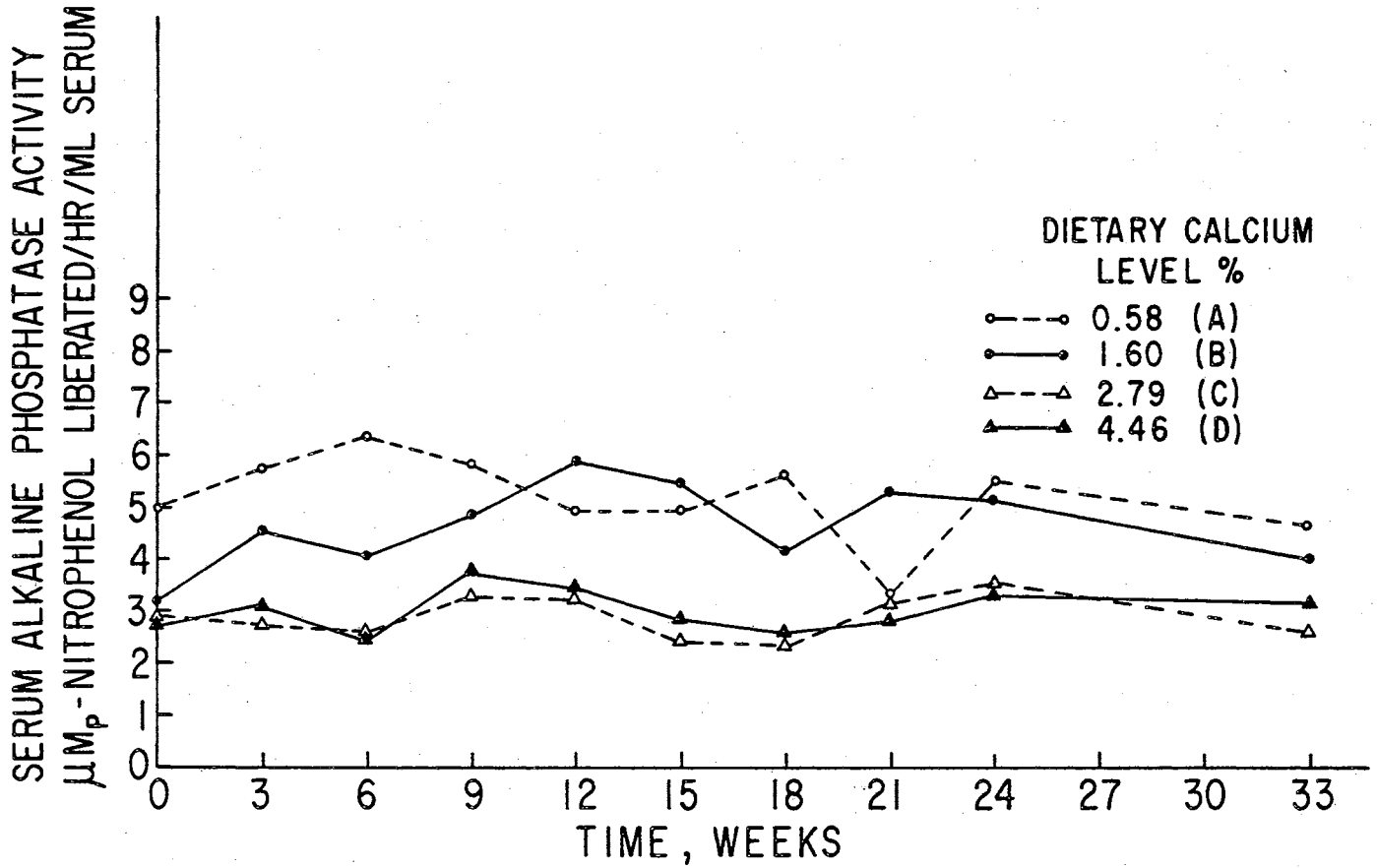

Figure 5. Serum Alkaline Phosphatase Activity of the Lambs Fed the Various Levels of Calcium. After Adjusting for Initial Differences, the Differences Among Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table X.

In addition, there appears to be some question relative to the origin of serum alkaline phosphatase. Yong (1967) reported that adult human serum contains predominantly liver alkaline phosphatase and little or no bone alkaline phosphatase. Saini and Posen (1969) noted that the rise in rat serum alkaline phosphatase activity after feeding appeared to be the result of entry of intestinal phosphatase into the blood circulation.

In experiments with baby pigs, Washam (1968) reported that serum alkaline phosphatase activity decreased as baby pigs became older, and that the decrease was more rapid as the dietary level of calcium and phosphorus was increased. Long, Ullrey and Miller (1965) noted that serum alkaline phosphatase activity declined during the interval from birth to 4 weeks of age. In contrast, Young et al. (1966) observed an increase in serum alkaline phosphatase activity as wether lambs increased in age from approximately 4 to 9 months. It was observed that when the lambs were fed diets low in phosphorus with adequate calcium and vitamin D, the low dietary phosphorus level had no effect upon alkaline phosphatase activity. These results may be due to species differences, differences in the origin of serum alkaline phosphatase or other factors. What these results mean in terms of calcium metabolism is not clear. Thus, the use of serum alkaline phosphatase activity to reflect bone growth or calcification may be questionable.

Hematocrit and Hemoglobin Levels

A general depression of hematocrit and hemoglobin concentration occurred during the initial 9 weeks (Figures 6 and 7, respectively). Following this depression, the hematocrit tended to remain relatively

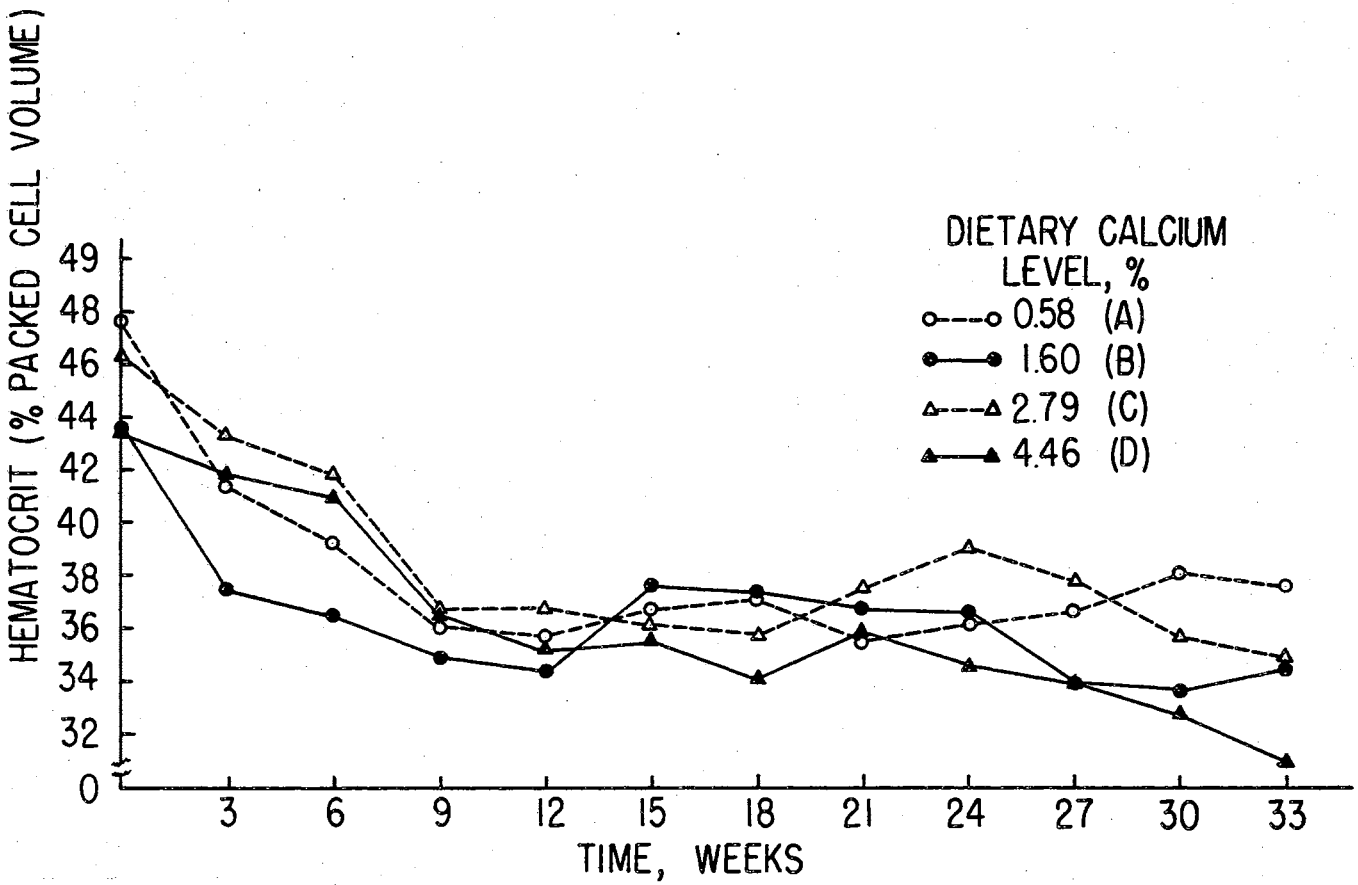

Figure 6. The Hematocrit of the Blood of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XII.

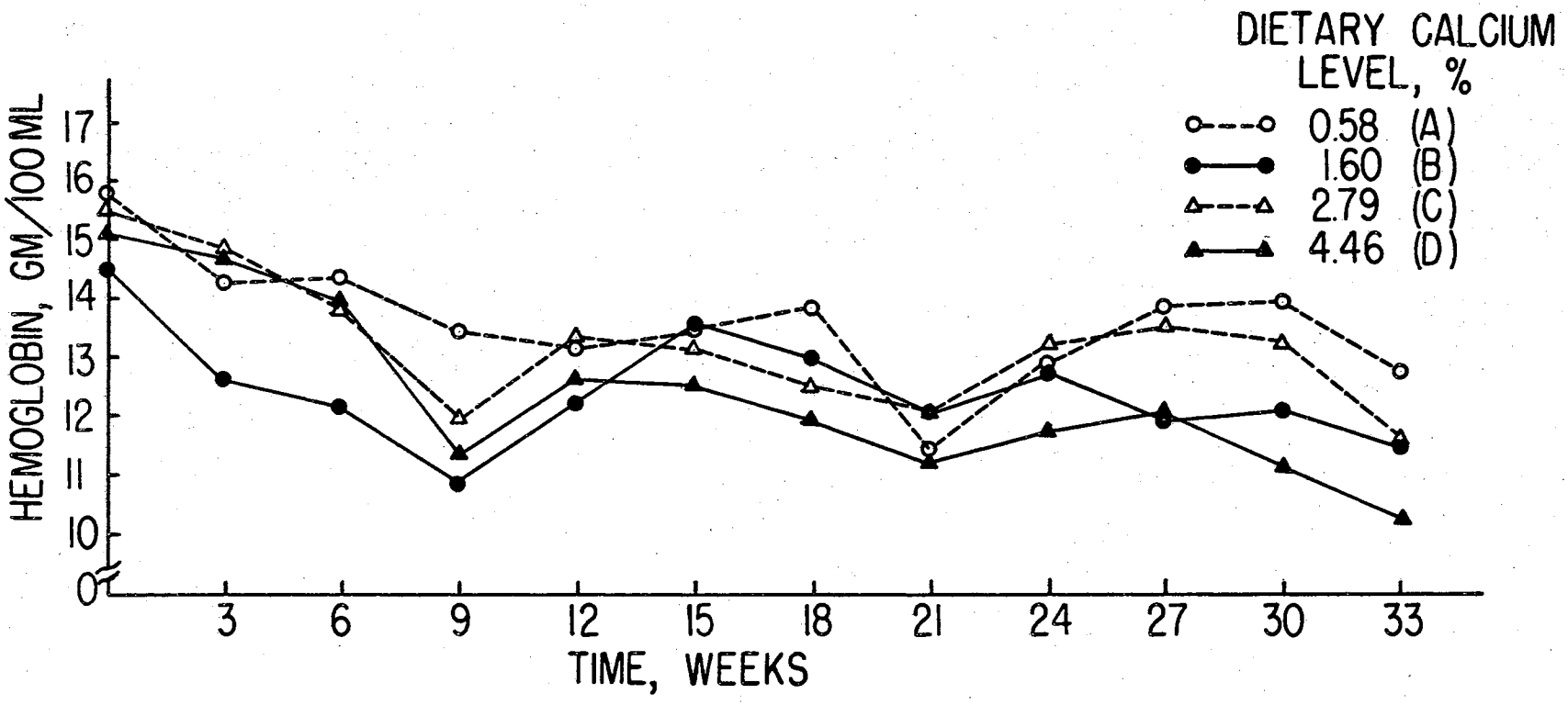

Figure 7. The Concentration of Hemoglobin in the Blood of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XI.

constant. No statistically significant treatment differences were observed in the hematocrit and hemoglobin concentrations. The mean hematocrit and hemoglobin levels were slightly above the normal ovine (3 to 12 months of age) levels reported by Ullrey et al. (1965). Swenson, Goetsch and Underbjerg (1962) observed no differences in hematocrit and hemoglobin concentration when Hereford heifers were fed excess calcium with a calcium to phosphorus ratio of 10:1.

Mineral Balance

The ingestion, excretion and retention patterns of calcium are illustrated in Figure 8. Similar patterns were observed for the amount ingested, the amount excreted via the feces and that retained. As the quantity of calcium ingested increased, the amount excreted via the feces and the amount retained increased significantly ($P < .01$). These results tend to agree with early work conducted with dairy heifers by Lindsey (1931), as cited by Duncan (1958). Lueker and Lofgreen (1961) observed that the amount of calcium absorbed by sheep was directly related to the amount fed, and that the metabolic fecal loss of calcium was independent of the amount absorbed. Using dairy cows, Manston (1967) found that endogenous calcium loss increased slightly with increased absorption. Nevertheless, the calcium balance did increase as the amount absorbed increased. These results tend to agree with those observed in this experiment.

Inasmuch as the levels of phosphorus, magnesium, copper and zinc were approximately the same in each treatment, differences observed in the amounts ingested corresponded to differences in feed consumption. Though not statistically significant, feed consumption tended to

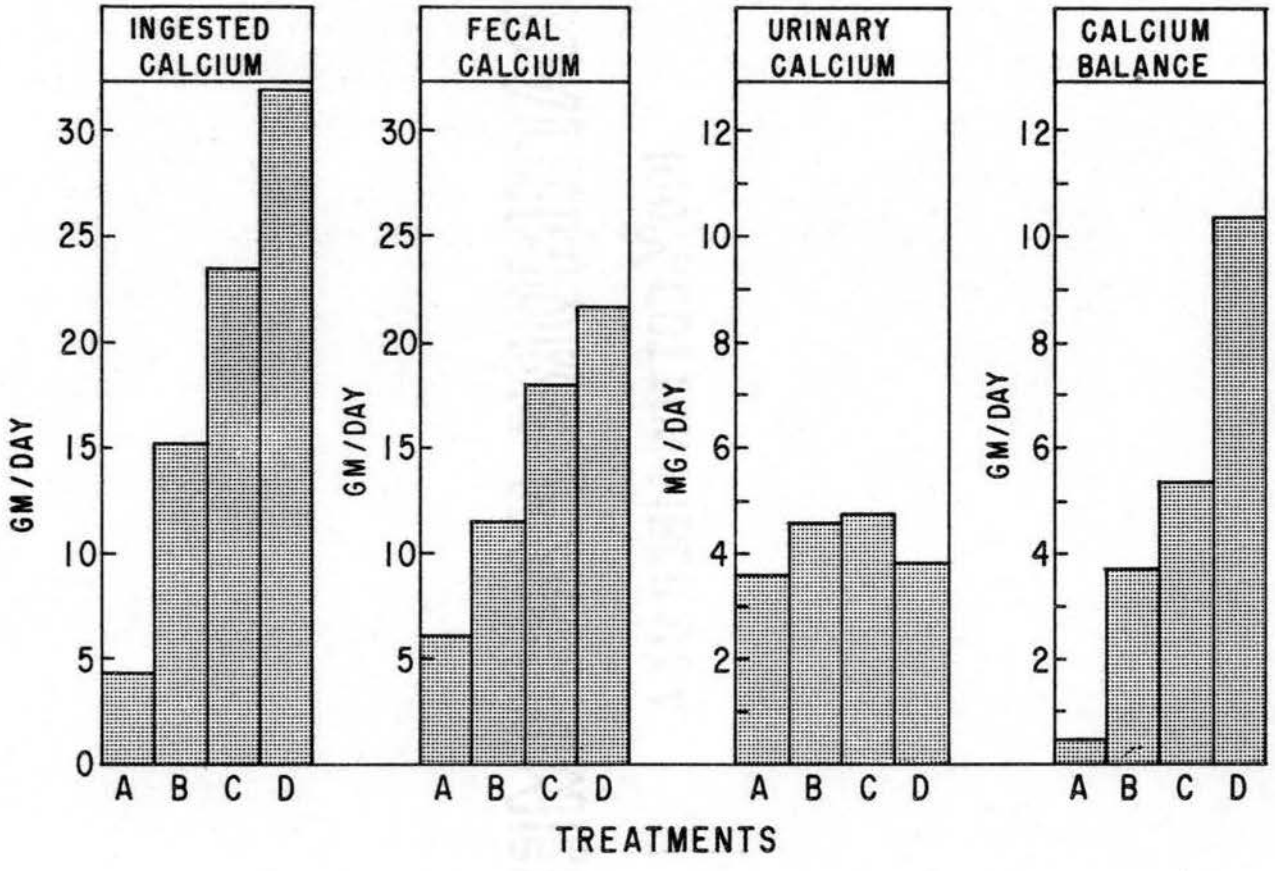

Figure 8. The Amount of Calcium Ingested, Excreted and Retained by the Lambs Fed the Various Levels of Calcium. The Differences in the Amount Ingested, Excreted via the Feces and in the Amount Retained were Significant ($P < .01$). These Data are Presented Numerically in Appendix Tables XIII, XIV, XV and XVI. (Treatments, i.e., dietary levels of calcium are: A=0.58%; B=1.60%; C=2.79%; D=4.46%)

decrease in the following order during the balance phase: B, C, A and D.

The ingestion, excretion and retention patterns of phosphorus, magnesium, copper and zinc are represented in Figures 9, 10, 11 and 12, respectively. The amount of calcium ingested did not influence the excretion or retention of phosphorus, magnesium and copper. As the dietary level of calcium increased to 2.79%, the retention of zinc tended to increase. At the highest level of dietary calcium, however, a slightly negative zinc retention was observed. It is the opinion of the author that this may not represent a real treatment effect in that the amount of zinc ingested was difficult to determine accurately due to difficulty encountered in obtaining feed samples containing representative quantities of zinc. Considerable between feed sample variation was observed for zinc.

Serum Minerals

Serum calcium levels observed during the balance and growth phases are portrayed graphically in Figure 13. No statistically significant differences existed among the overall treatment means (Appendix Table XXXII). The levels of serum calcium tended to decrease during the initial 9 weeks on experiment with no apparent treatment trends. All serum calcium levels were within the normal range of 9 to 12 mg/100 ml as reported by Dukes (1955), and just slightly below the normal mean value for sheep reported by Long et al. (1965b). High levels of dietary calcium did not appear to influence the level of serum calcium (Figure 13). This is in agreement with the results of experiments with cattle, conducted by Dowe et al. (1957), Wise et al. (1963) and Swenson et al.

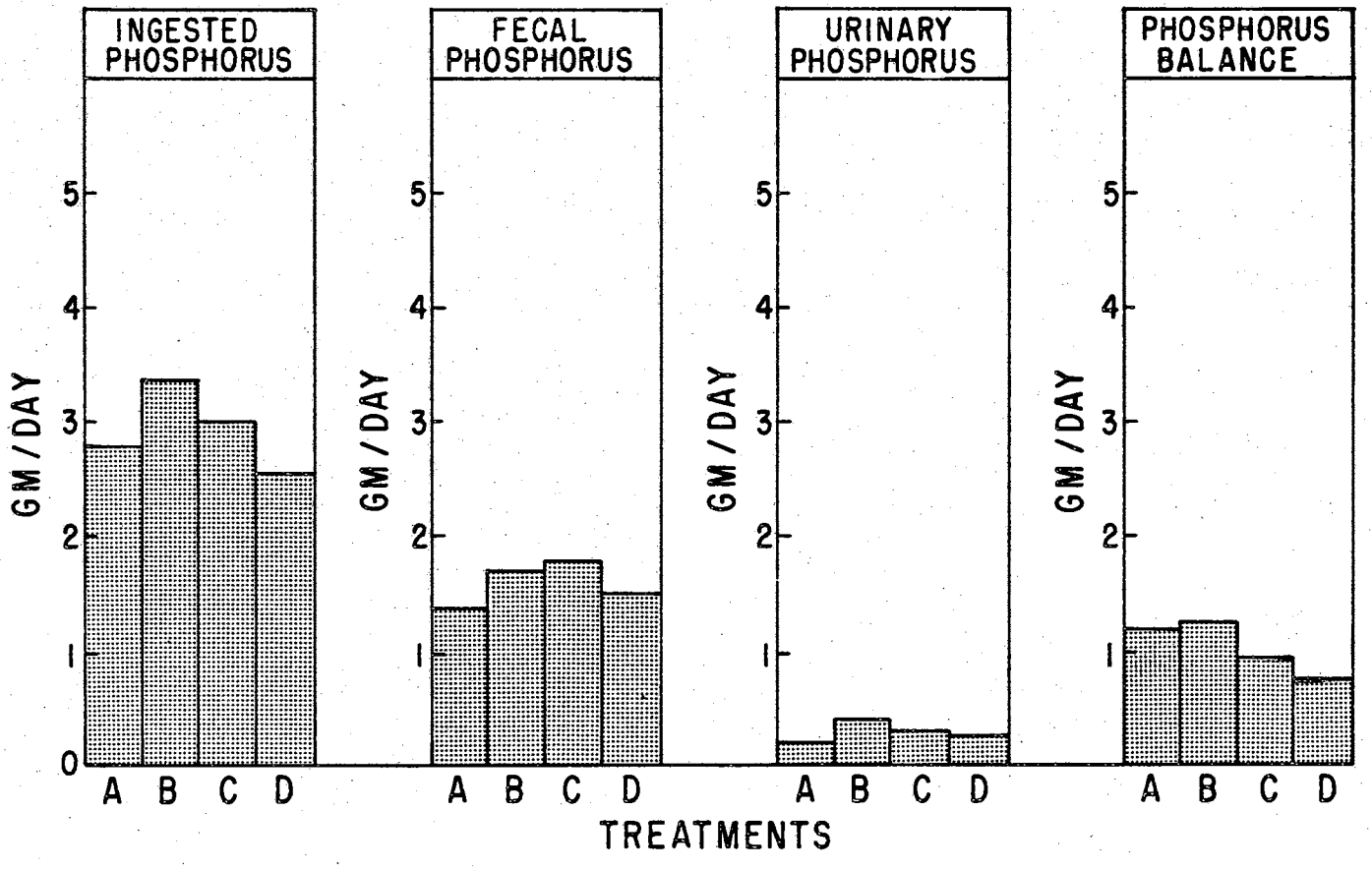

Figure 9. The Amount of Phosphorus Ingested, Excreted and Retained by the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Tables XVII, XVIII, XIX and XX. (Treatments, i.e., dietary levels of calcium are: A=0.58%; B=1.60%; C=2.79%; D=4.46%)

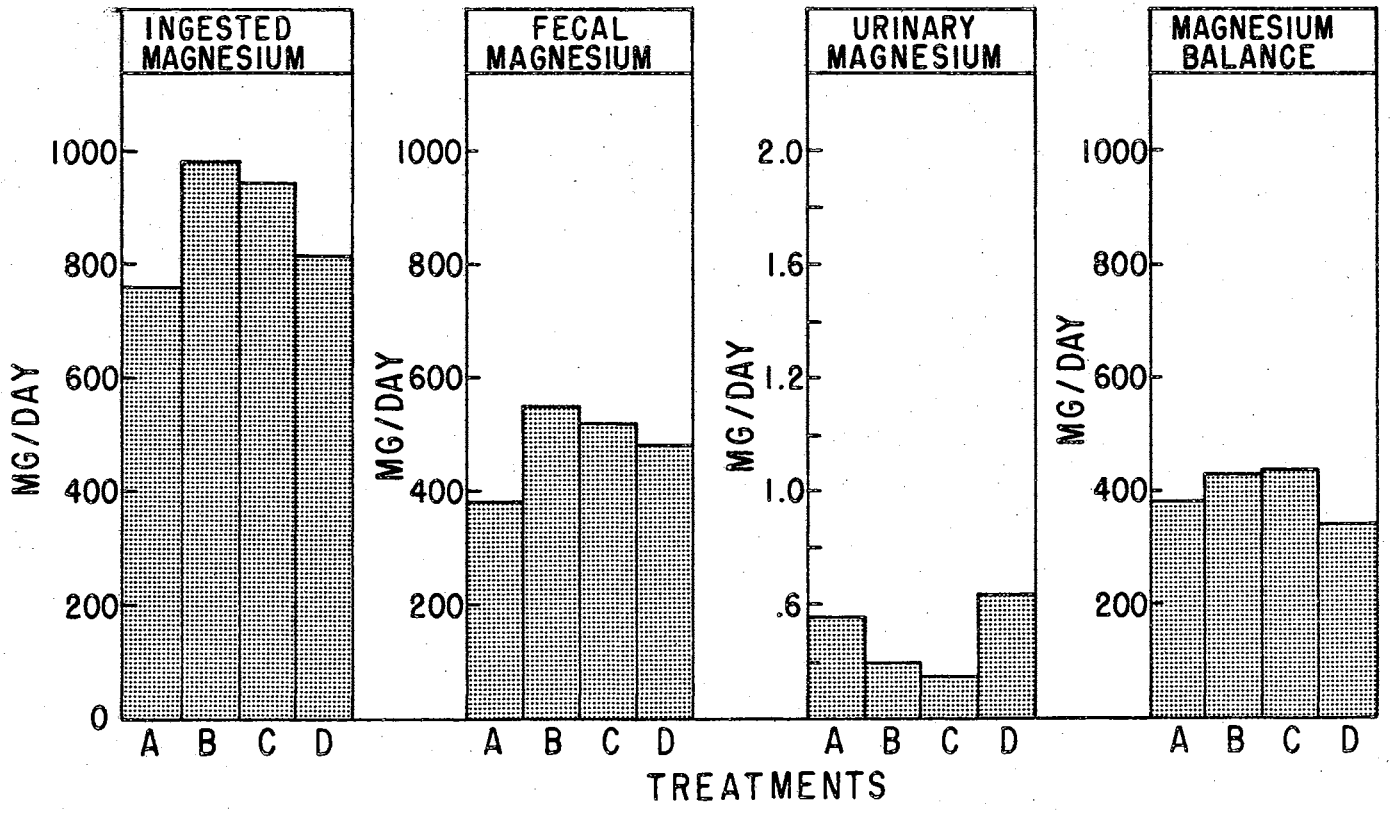

Figure 10. The Amount of Magnesium Ingested, Excreted and Retained by the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means Were Not Statistically Significant. These Data are Presented Numerically in Appendix Tables XXI, XXII, XXIII and XXIV. (Treatments, i.e., dietary levels of calcium are: A=0.58%; B=1.60%; C=2.79%; D=4.46%)

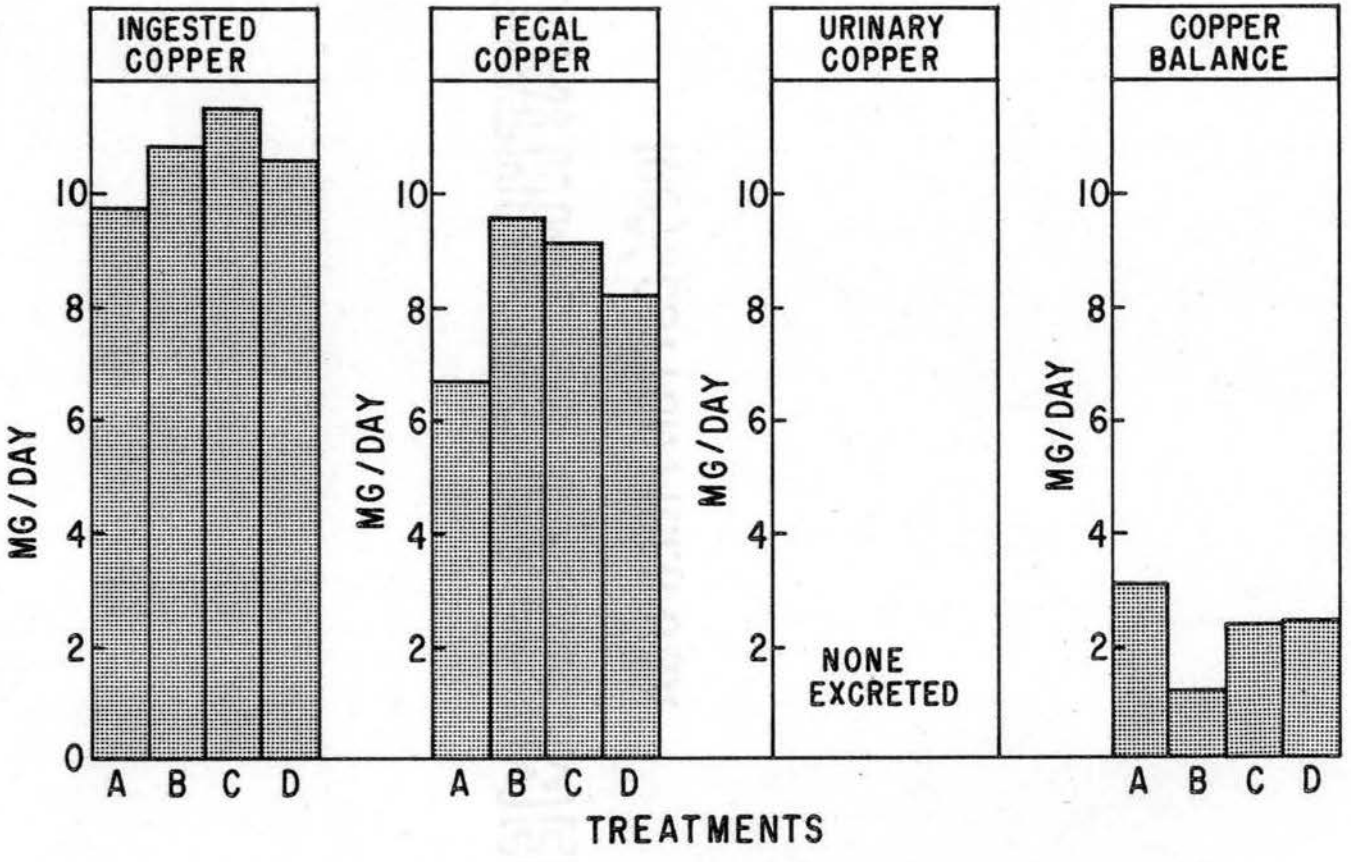

Figure 11. The Amount of Copper Ingested, Excreted and Retained by the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Tables XXV, XXVI and XXVII. (Treatments, i.e., dietary levels of calcium are: A=0.58%; B=1.60%; C=2.79%; D=4.46%)

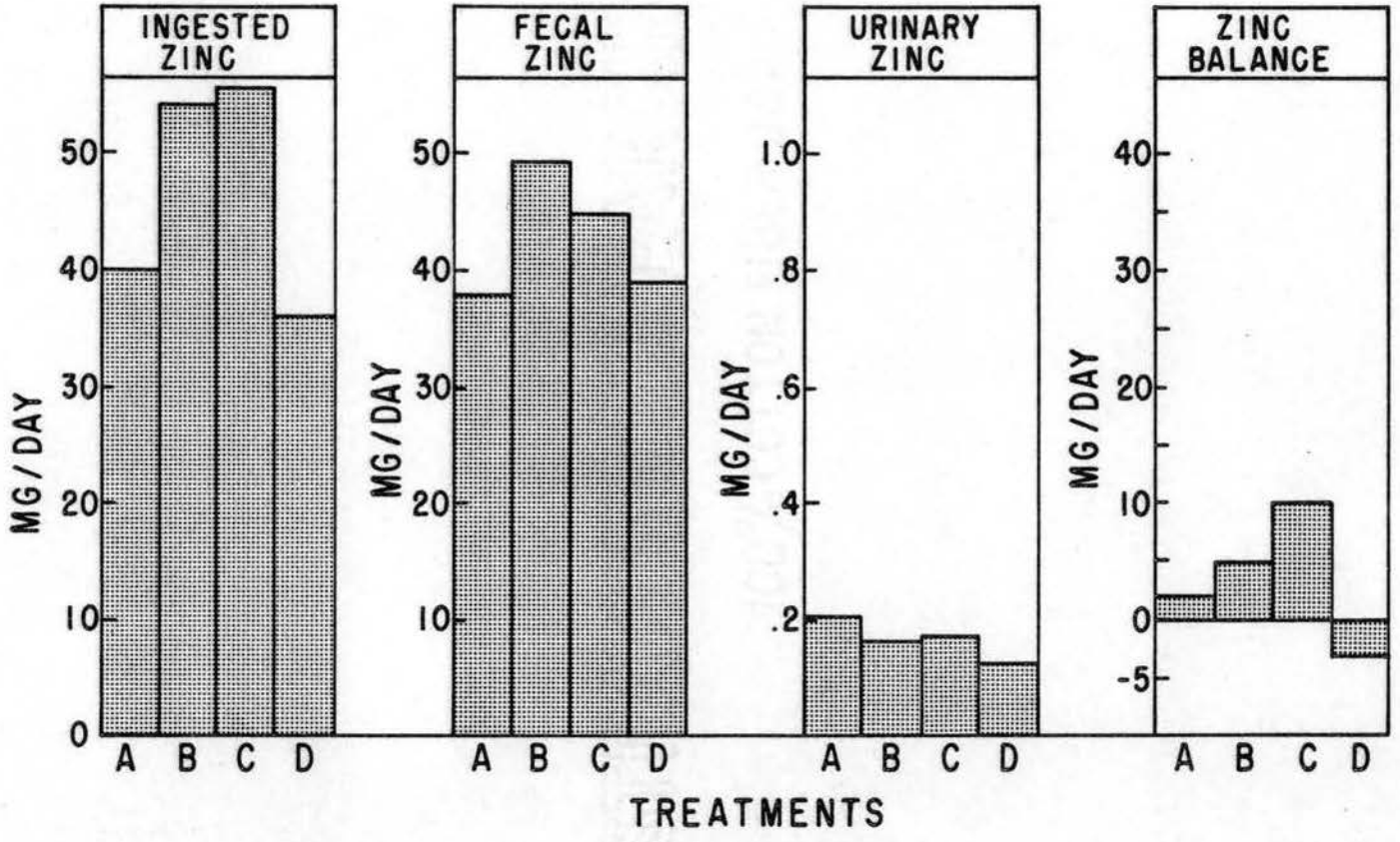

Figure 12. The Amount of Zinc Ingested, Excreted and Retained by the Lambs Fed the Various Levels of Calcium. The Differences Among Treatment Means in the Amount Retained were Significant ($P < .01$). These Data are Presented Numerically in Appendix Tables XXVIII, XXIX, XXX and XXXI. (Treatments, i.e., dietary levels of calcium are: A=0.58%; B=1.60%; C=2.79%; D=4.46%)

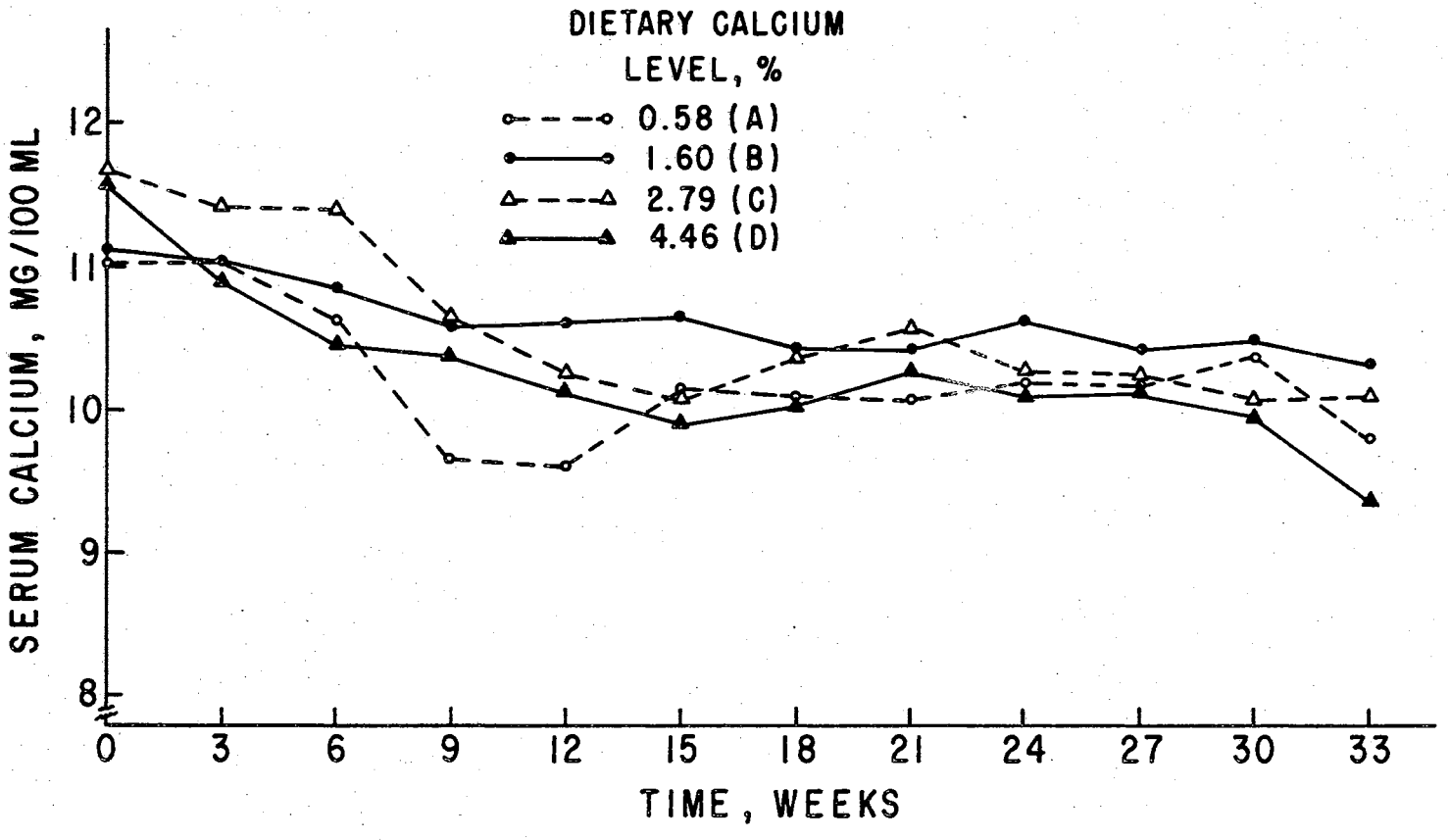

Figure 13. The Concentration of Calcium in the Serum of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XXXII.

(1962). When one considers the homeostatic mechanisms regulating serum calcium level, these results are not surprising.

As illustrated in Figure 14, very erratic serum phosphorus levels were observed. Though irregular, all values were within the normal range of 3 to 8 mg/100 ml reported by Dukes (1955). The level of significance among the overall treatment means was 0.294 (Appendix Table XXXIII). It appears that the lambs on the lowest level of dietary calcium (0.58%) tended to have the highest serum phosphorus levels.

Previous work indicates that the inorganic phosphorus levels of serum can reflect the phosphorus status of lambs (Preston and Pfander, 1964; Young et al., 1966). Results of this experiment (Figure 14) tend to agree with those of Dowe et al. (1957), Swenson et al. (1962) and Wise et al. (1963). These researchers fed rations with high calcium to phosphorus ratios, with adequate phosphorus, to growing cattle. It was observed that high levels of dietary calcium had little or no effect on plasma inorganic phosphorus levels. Smith, Holck and Spafford (1966) and Wise et al. (1963) observed that the serum inorganic phosphorus levels of cattle were directly related to the level of dietary phosphorus.

Serum magnesium values are illustrated graphically in Figure 15 and numerically in Appendix Table XXXIV. The overall mean treatment concentrations of serum magnesium (Appendix Table XXXIV) tended to decrease as the level of dietary calcium was increased. Levels of 2.788, 2.624, 2.588 and 2.540 mg/100 ml were observed for treatments A, B, C and D, respectively. The level of significance among the overall mean concentrations was 0.11.

Work by Cramer and Dueck (1962) with dogs, and that conducted with

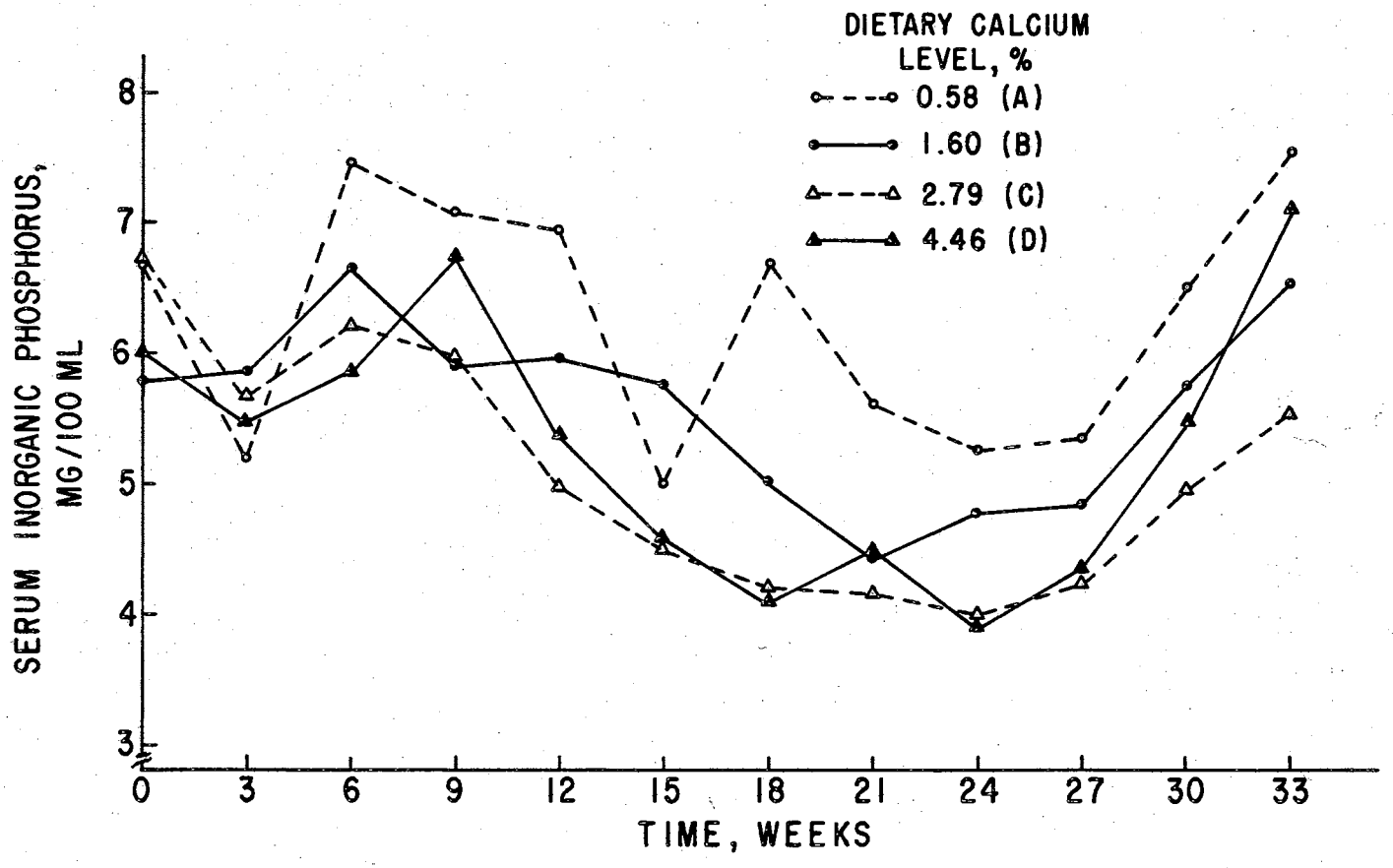

Figure 14. The Concentration of Inorganic Phosphorus in the Serum of the Lambs Fed the Various Levels of Calcium. The Level of Significance Among the Overall Treatment Means was 0.294. These Data are Presented Numerically in Appendix Table XXXIII.

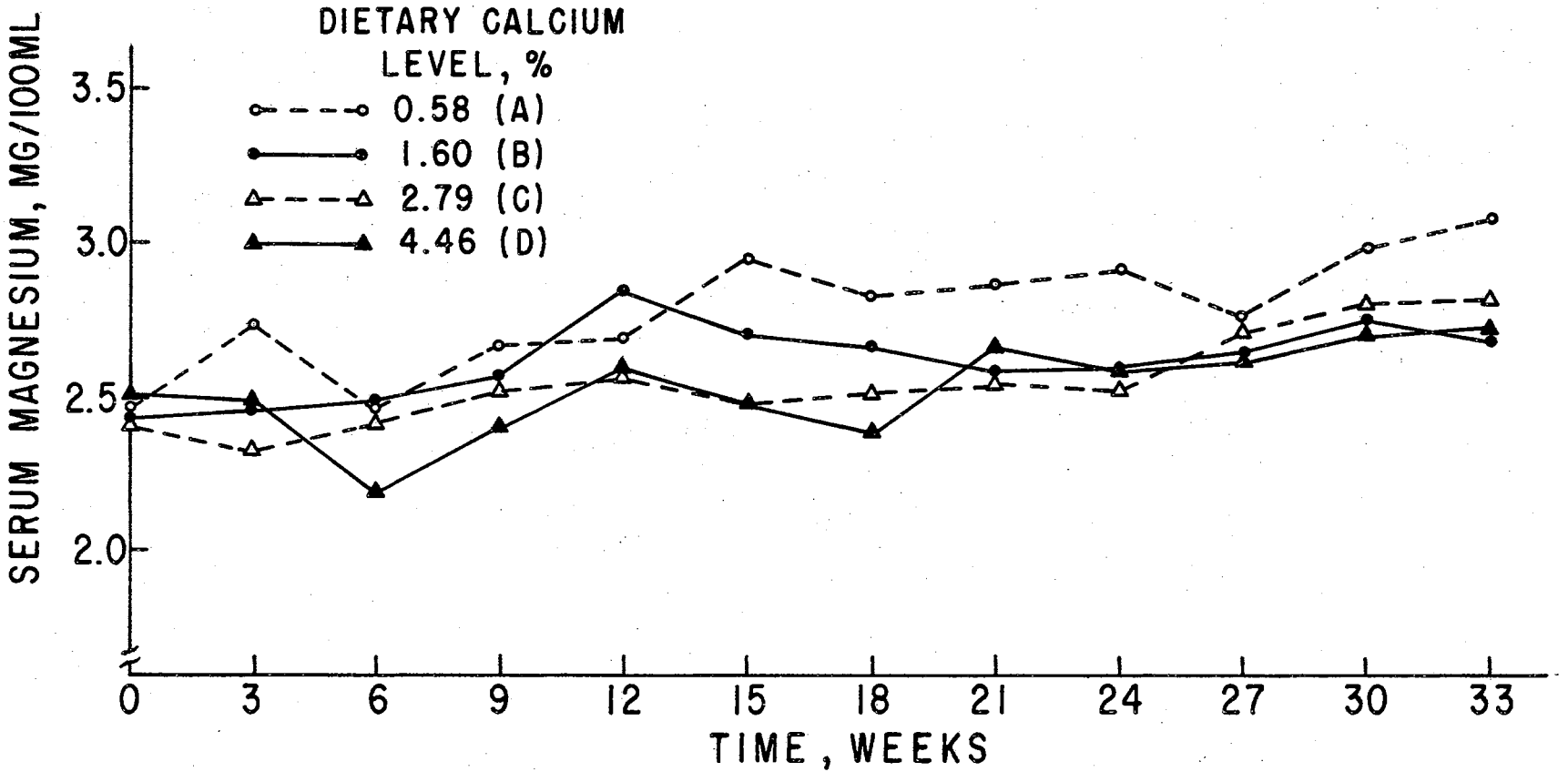

Figure 15. The Concentration of Magnesium in the Serum of the Lambs Fed the Various Levels of Calcium. The Level of Significance Among the Overall Treatment Means was 0.11. These Data are Presented Numerically in Appendix Table XXXIV.

sheep by Care and van't Klooster (1965) suggests that magnesium and calcium are absorbed by a common mechanism in the small intestine, distal to the duodenum. The major site of magnesium absorption in sheep appears to be distal to the duodenum (Care and van't Klooster, 1965; Field, 1961; Scott, 1965). Care and van't Klooster (1965) observed that the net absorption rate of magnesium tended to decrease as the concentration of calcium was increased in the mid-ileum of sheep. They demonstrated that increasing the calcium concentration resulted in an increase in the rate of calcium absorption. In addition to a common absorptive mechanism in the ileum, it has been suggested that high calcium concentrations may reduce magnesium availability in the ileum of the calf and *in vitro* (Smith and McAllan, 1966; Smith and McAllan, 1967). It appears that high levels of dietary calcium could interfere with the magnesium status of the ruminant animal.

Results of this experiment (Figure 15) indicate only a slight relationship between the dietary level of calcium and the level of magnesium in the serum. The overall treatment mean concentration of serum magnesium tended to decrease as the level of dietary calcium was increased. All serum magnesium levels were near those considered to be normal for growing lambs (Long *et al.*, 1965b).

No apparent treatment differences in serum copper levels were observed until the lambs had been on experiment at least 21 weeks (Figure 16). From this time until termination of the experiment, the serum copper levels of the lambs on the highest level of calcium decreased and were distinctly lower than the other treatment levels. There was a statistically significant difference ($P < .05$) among the overall treatment means (Appendix Table XXXV).

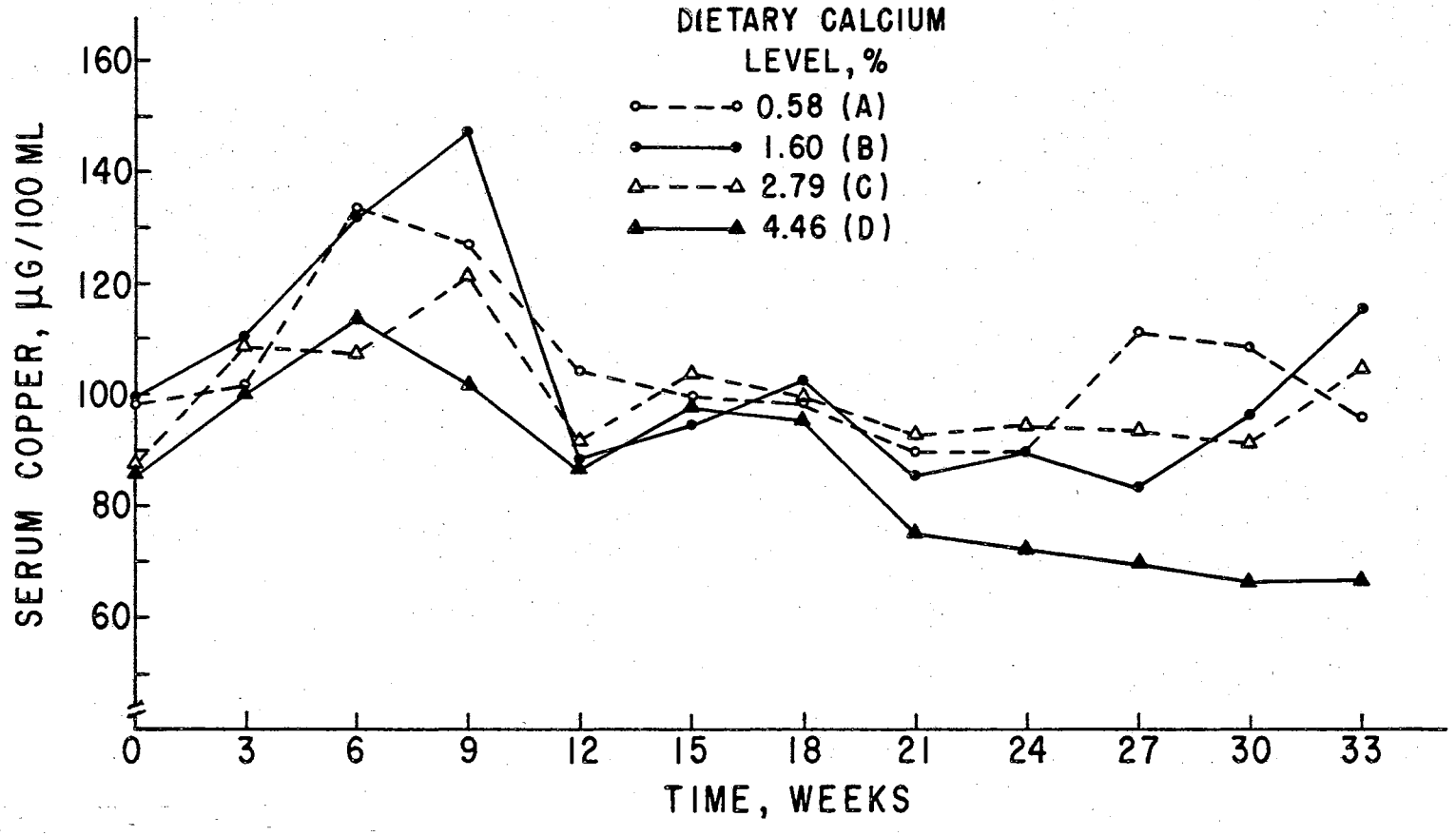

Figure 16. The Concentration of Copper in the Serum of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XXXV.

Wintrobe, Cartwright and Gubler (1953) observed that during a deficiency of copper in pigs, the reduction in plasma copper was much greater than the reduction in erythrocyte copper. Thus, plasma copper levels apparently can be used as an indicator of copper status and it appears to be a more reliable indicator than whole blood copper. Goodrich, Bradley and Tillman (1968) noted that lambs from 13 nutritional experiments had an overall mean initial plasma copper level of 111.0 μg/100 ml. Values observed in this experiment (Figure 16) were near this value with the exception of those lambs fed the highest level of dietary calcium during the final 12 weeks of the experiment. No previous work has been reported which indicates that high levels of dietary calcium may influence copper metabolism in sheep.

The dietary level of calcium did not appear to influence serum zinc levels (Figure 17 and Appendix Table XXXVI). Ott et al. (1965) determined that the zinc requirement of growing lambs fed a purified diet was from 18 to 33 ppm. The dietary level of zinc in this experiment was considerably above these levels. This could have influenced the results observed. Ott et al. (1965) observed that serum zinc levels of growing lambs decreased linearly as the dietary level of zinc decreased from 33 to 3 ppm. Similar results were observed by Mills and Dalgarno (1967). Thus, serum levels of zinc appear to reflect the dietary intake of zinc under certain conditions.

The effect of high dietary levels of calcium upon the absorption of zinc in ruminants has received very little attention. It has been observed that high levels of calcium accentuated zinc deficiency symptoms in pigs (Oberleas et al., 1962) and rats (Oberleas et al., 1966) when the diet contained phytate, however, this did not occur when phytate was

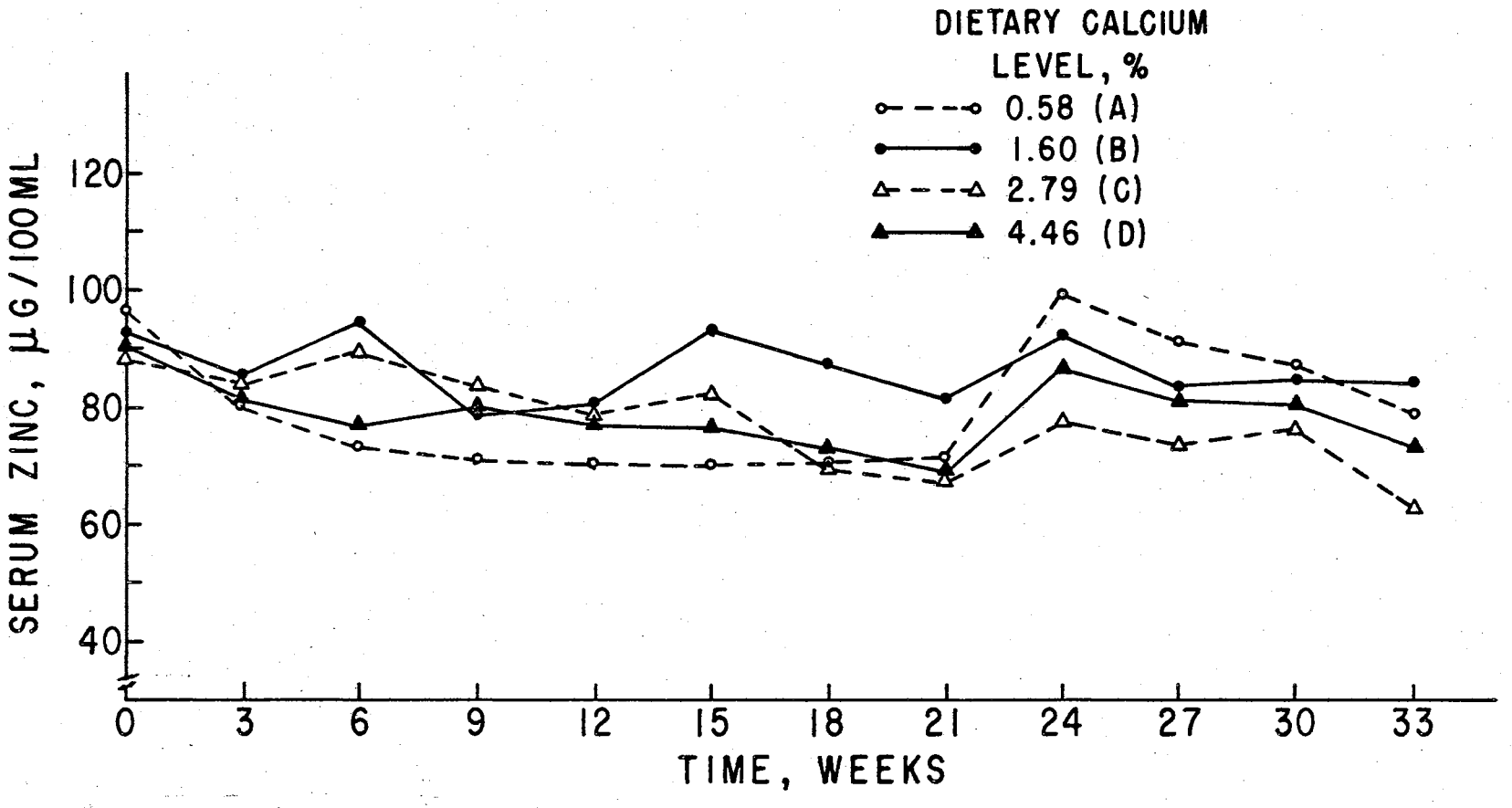

Figure 17. The Concentration of Zinc in the Serum of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XXXVI.

not present. Even if phytate were present in the ruminant diet, hydrolysis of phytate can occur in the rumen (Raun et al., 1956). Therefore, high levels of calcium in a ruminant diet, containing adequate zinc, would not be expected to cause a zinc deficiency by decreasing the availability of zinc. The results of this experiment (Figure 17) tend to substantiate these observations.

The levels of serum potassium were not influenced by the dietary levels of calcium (Figure 18). No statistically significant differences existed among the overall treatment mean concentrations (Appendix Table XXXVII). Serum potassium levels were similar to the average of 25.3 mg/100 ml reported by Long et al. (1965b) and the average of 23.2 mg/100 ml determined by Field, Wiener and Wood (1969) for sheep.

Wool Minerals

The concentrations of various mineral elements in clean dry wool were determined primarily to aid in monitoring the possible effects of high levels of dietary calcium upon the metabolism of the elements involved. If wool could be used to monitor the mineral element status of an animal, it would have advantages over other tissues in that samples would be easy to obtain without harm to the animal.

The percent ash of the wool appeared to be somewhat irregular (Figure 19 and Appendix Table XXXVIII). It is the opinion of the author that a portion of the irregularity may possibly be attributed to the cleaning procedure utilized. The overall treatment mean ash content of the wool tended to increase as the level of dietary calcium increased. The level of significance among the overall treatment means was approximately 0.20.

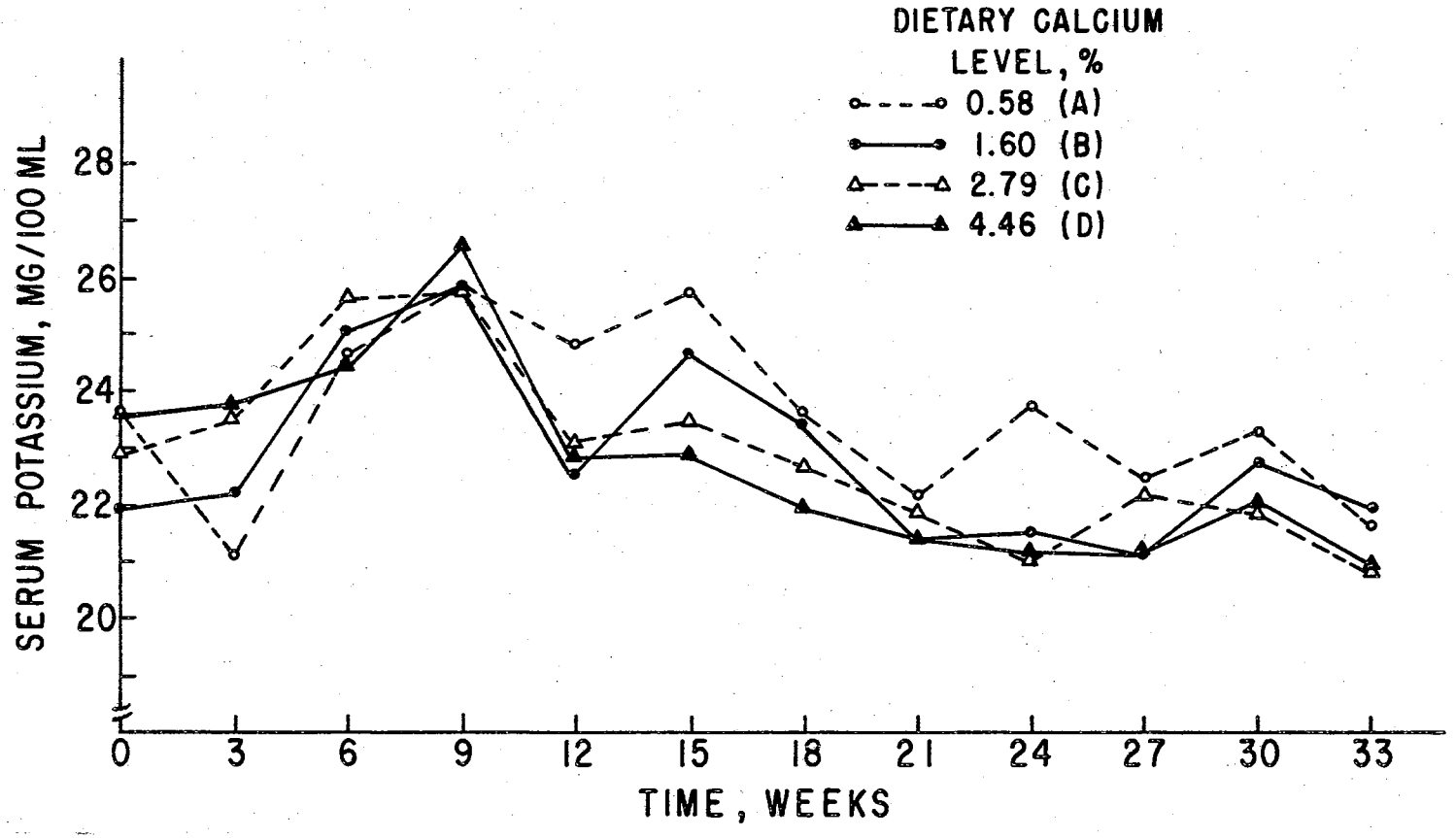

Figure 18. The Concentration of Potassium in the Serum of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XXXVII.

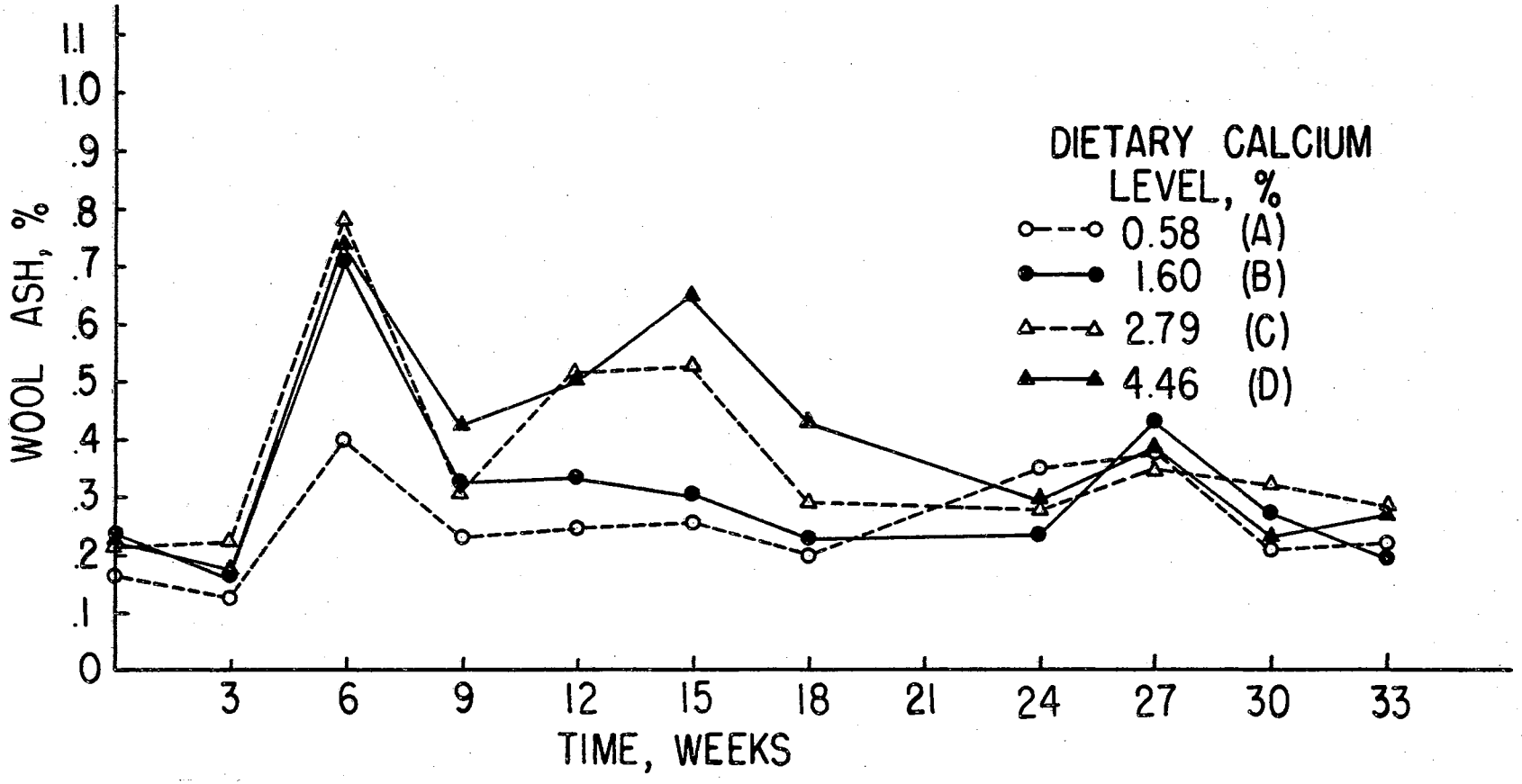

Figure 19. The Ash Content of the Wool of the Lambs Fed the Various Levels of Calcium. The Level of Significance Among the Overall Treatment Means was 0.20. These Data are Presented Numerically in Appendix Table XXXVIII.

The concentration of calcium in the wool was somewhat variable with time (Figure 20). Overall mean concentrations of 432, 645, 959 and 1005 ppm were observed for treatments A, B, C and D, respectively (Appendix Table XXXIX). Thus, as the level of dietary calcium increased, the concentration of calcium in the wool increased ($P < .05$). These data agree with the retention data in that as the dietary level of calcium increased, the retention of calcium increased.

Wool phosphorus concentrations were somewhat irregular during the initial weeks of the experiment (Figure 21), following this initial period it appeared that the concentration of phosphorus tended to increase as the level of dietary calcium increased. The level of significance among the overall treatment means was approximately 0.30 (Appendix Table XL).

Wool concentrations of magnesium, copper and zinc are presented graphically in Figures 22, 23, and 24, respectively. No treatment trends or statistically significant differences were observed.

The potassium content of the wool was somewhat erratic across time (Figure 25). A significant difference existed among the overall treatment means (Appendix Table XLIV). On the basis of the overall treatment means, it appeared that as the dietary level of calcium increased, the level of potassium in the wool tended to decrease.

Burns et al. (1964) conducted mineral analyses on fleeces from widely distributed growing areas in the United States and Australia. Much between fleece variation was observed for all elements studied, with the exception of phosphorus, sulfur and boron. In comparing the mineral element concentrations of the wool in this experiment to those observed by Burns et al. (1964), relatively larger values were observed

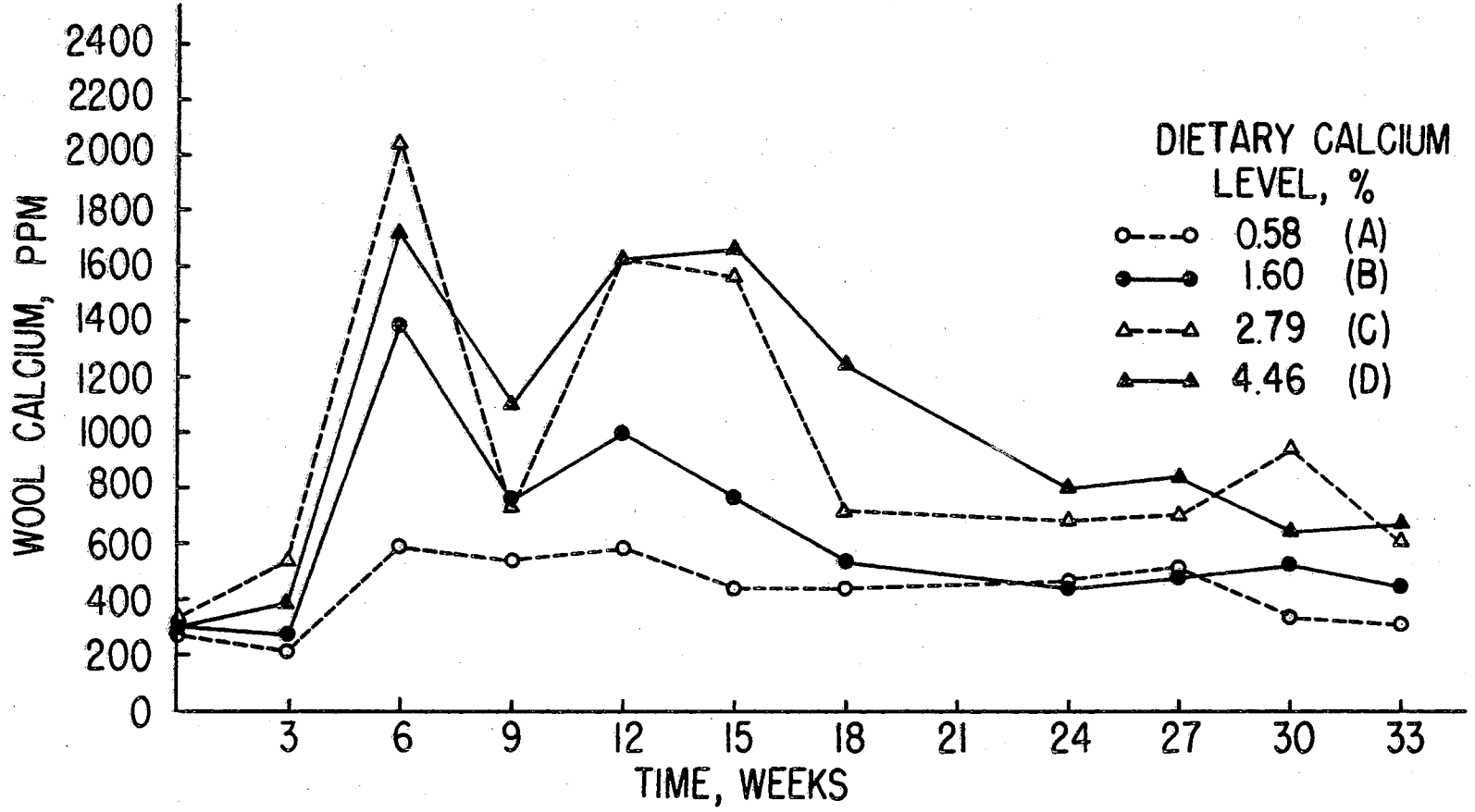

Figure 20. The Concentration of Calcium in the Wool of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were Significant ($P < .05$). These Data are Presented Numerically in Appendix Table XXXIX.

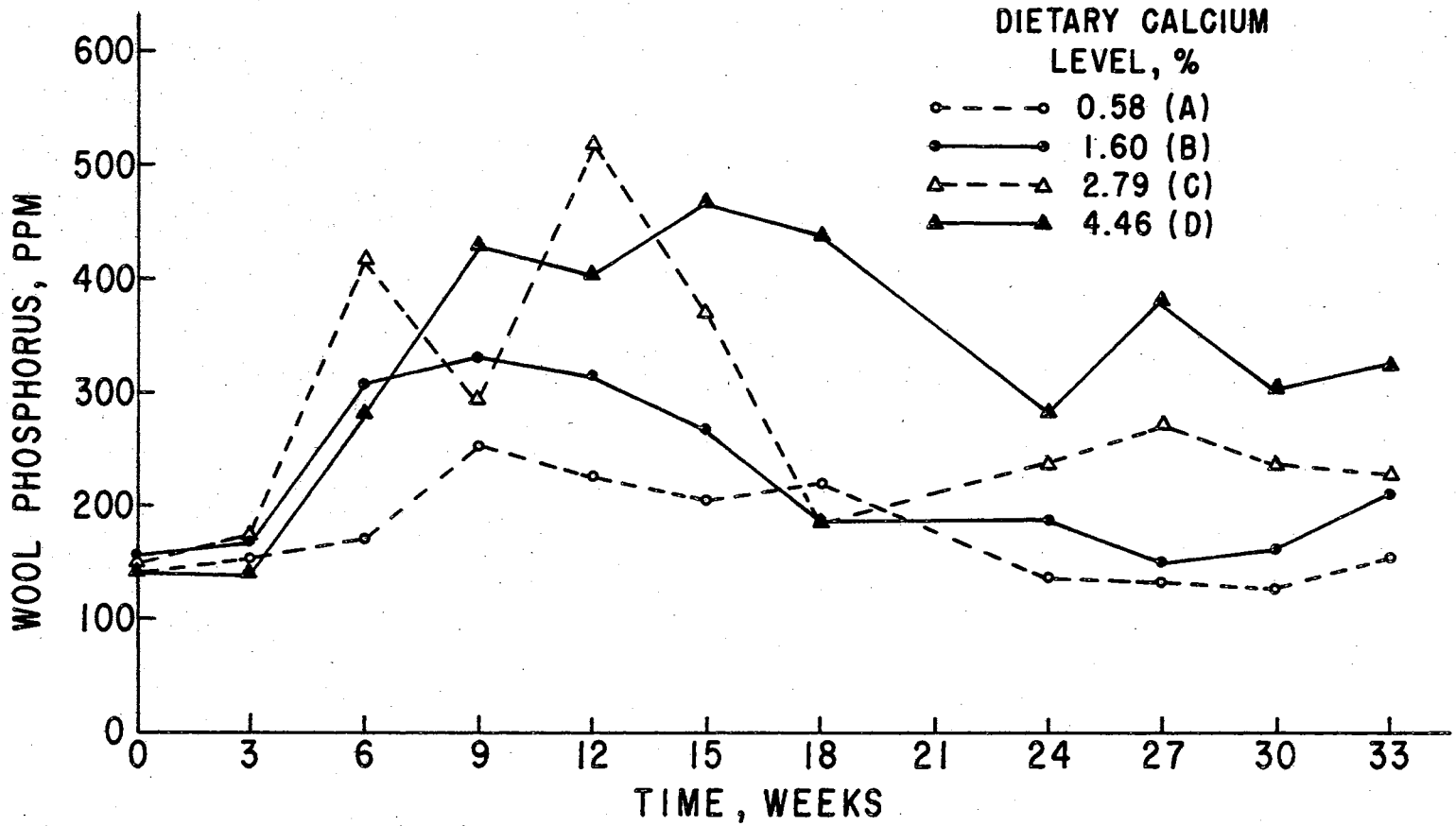

Figure 21. The Concentration of Inorganic Phosphorus in the Wool of the Lambs Fed the Various Levels of Calcium. The Level of Significance Among the Overall Treatment Means was 0.30. These Data are Presented Numerically in Appendix Table XL.

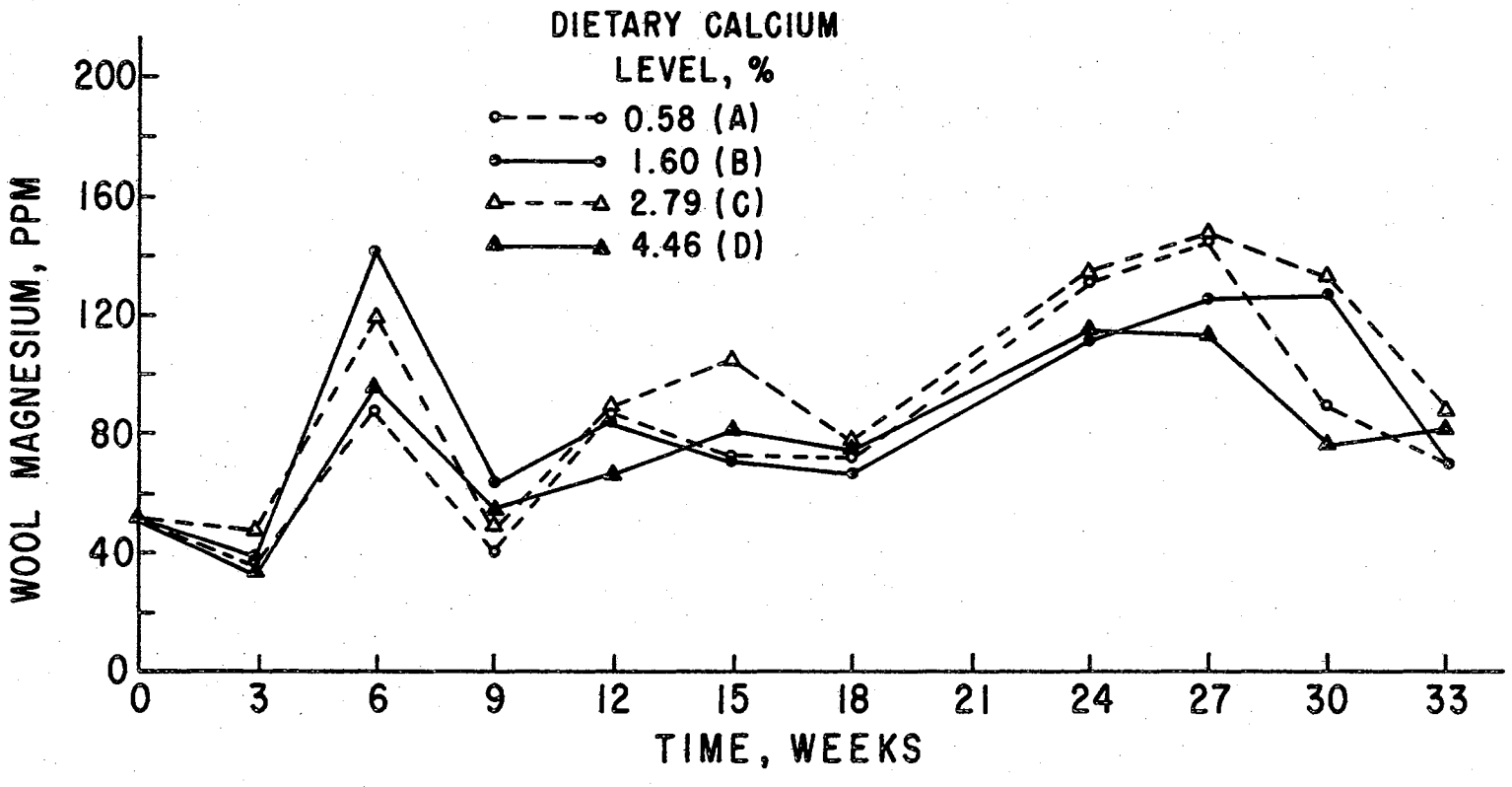

Figure 22. The Concentration of Magnesium in the Wool of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XLI.

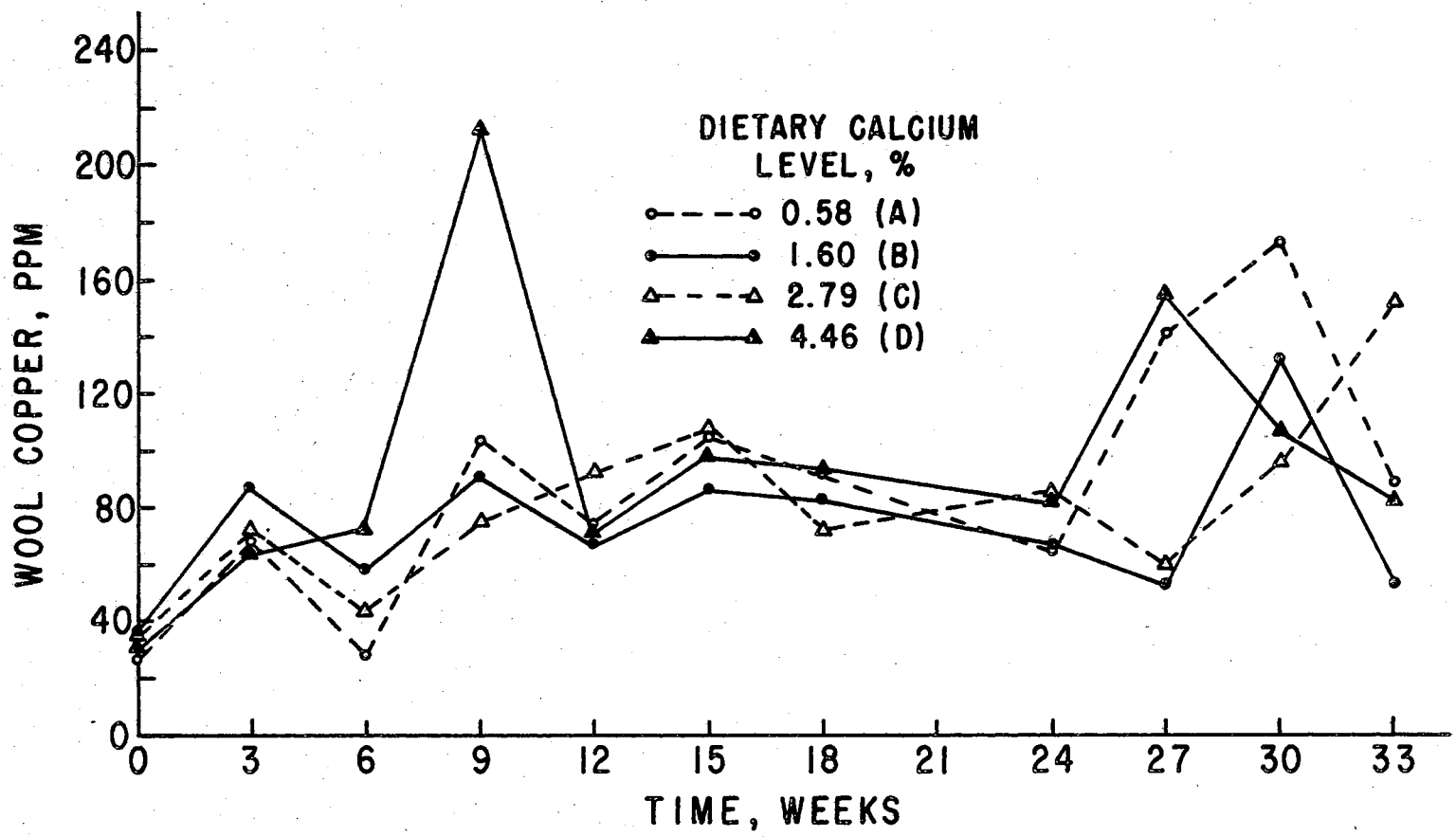

Figure 23. The Concentration of Copper in the Wool of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XLII.

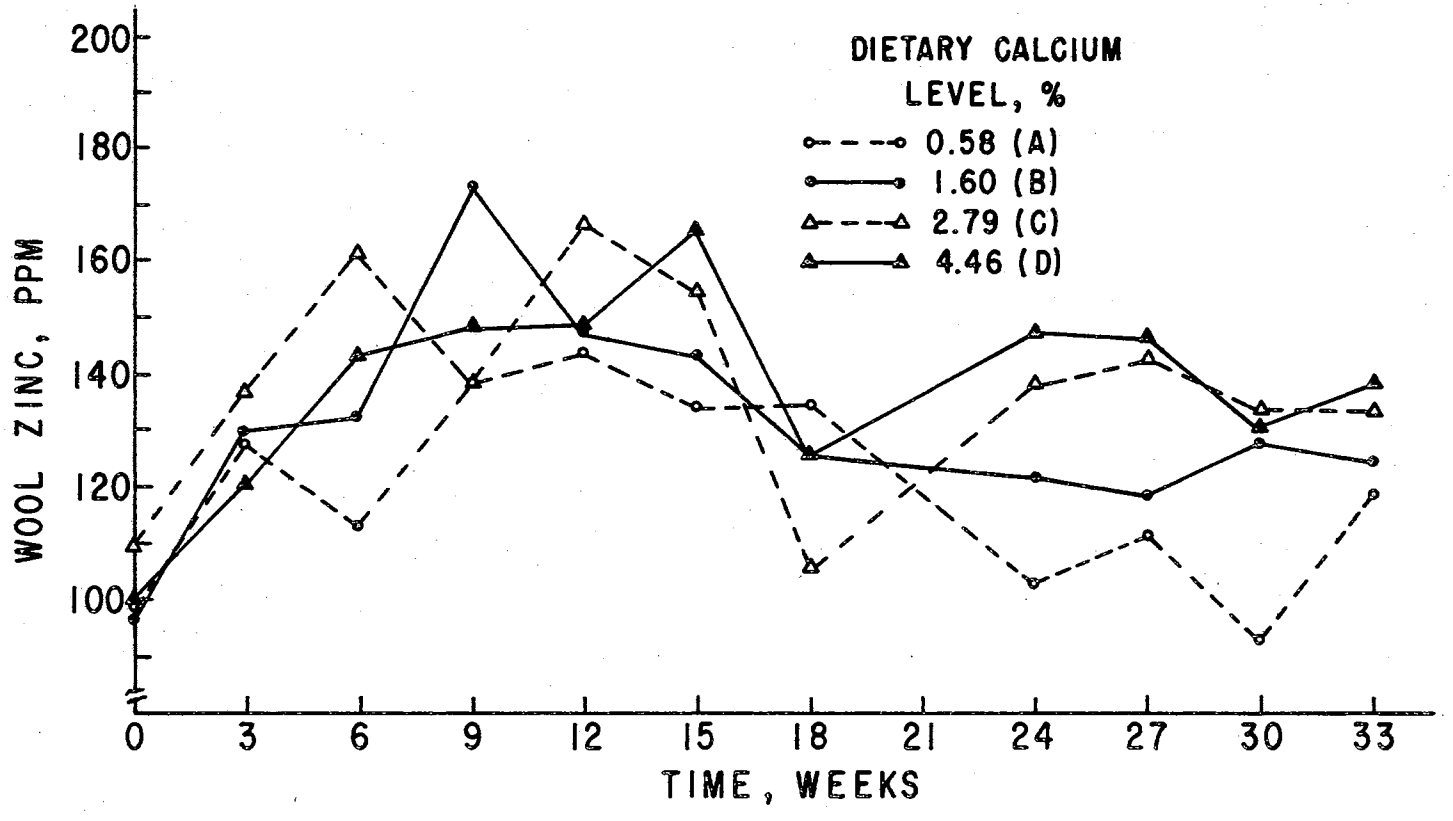

Figure 24. The Concentration of Zinc in the Wool of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were not Statistically Significant. These Data are Presented Numerically in Appendix Table XLIII.

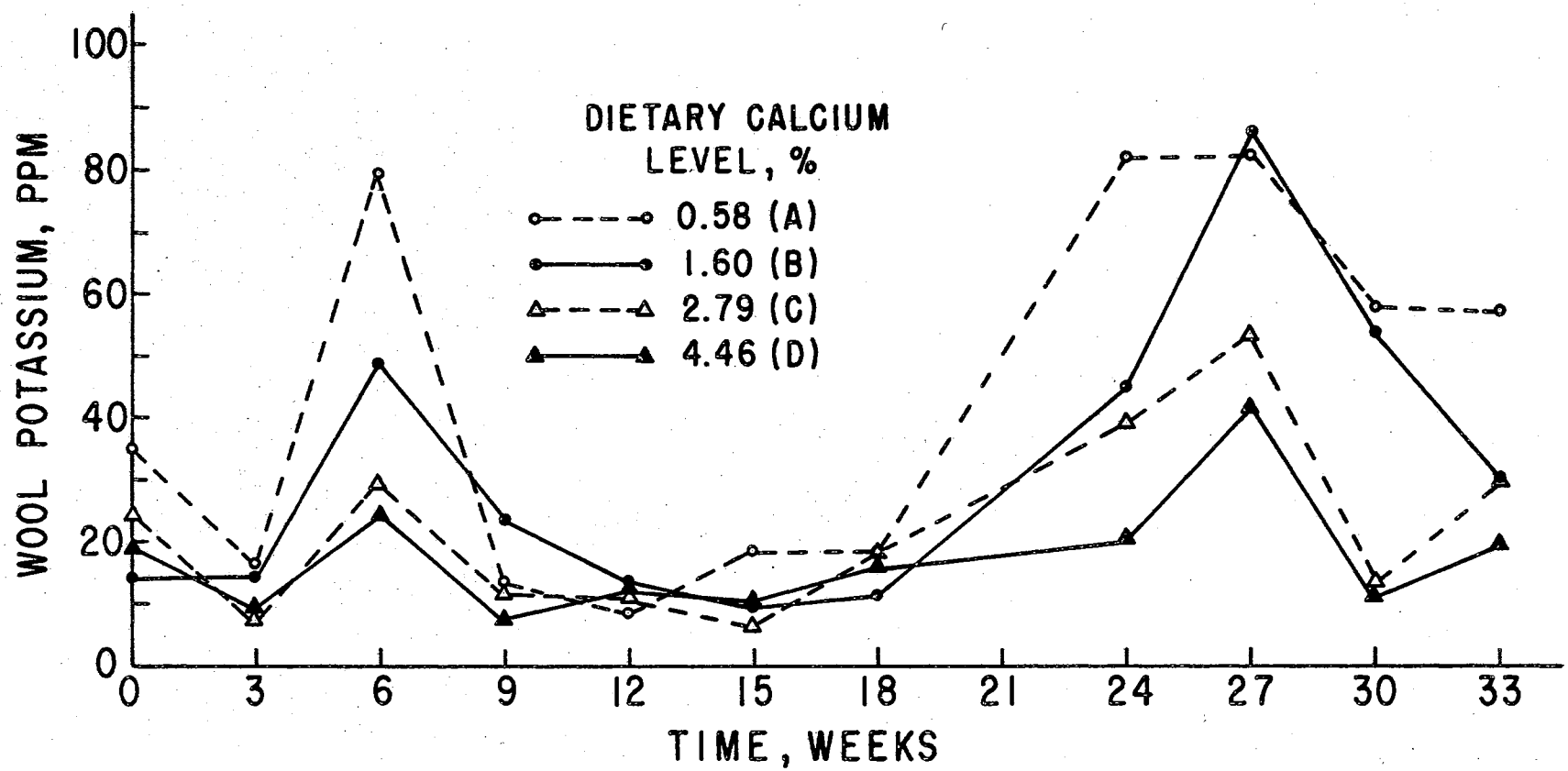

Figure 25. The Concentration of Potassium in the Wool of the Lambs Fed the Various Levels of Calcium. The Differences Among Overall Treatment Means were Significant ($P < .01$). These Data are Presented Numerically in Appendix Table XLIV.

in the present experiment for phosphorus, copper and zinc; a lower concentration of calcium with similar values for magnesium and potassium.

Considering the variation observed within treatments and with time for the elements evaluated in this experiment, the value of using wool concentrations as a means of monitoring mineral element status of lambs could certainly be questioned. It could possibly be of value if used in conjunction with wool growth and tissue levels.

Miller et al. (1965) observed that hair from zinc-deficient calves contained less zinc than that from calves given adequate zinc. Reinhold, Kfoury and Arslanian (1968) observed similar results with rats, however, lower zinc concentrations in hair could not be associated with decreased growth. In human studies involving trace elements, Schroeder and Nason (1969) concluded that concentrations of trace elements in hair may not reflect tissue reserves under normal conditions. Thus, the use of wool or hair concentrations of many elements may be of little value in estimating body reserves or monitoring the mineral status of an animal.

Alimentary Tract pH

The dietary level of calcium had no effect upon the pH of the digesta from the various segments of the alimentary tract of the anesthetized lambs (Table III). Storry (1961a) observed pH values similar to those found in this experiment (Table III) for the rumen, cecum and colon of sheep fed natural diets. However, Storry (1961a) observed lower values in the small intestine and higher values in the abomasum. The pH range of abomasal samples obtained in this experiment were very similar to those observed by Ash (1961). However, the pH of the digesta

TABLE III

THE pH OF VARIOUS PARTS OF THE ALIMENTARY TRACT OF THE ANESTHETIZED LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, % A 0.58	B 1.60	C 2.79	D 4.46	SE[a]
No. of lambs	3	3	3	3	
Rumen	5.65	5.73	5.39	5.70	.23
Abomasum	2.20	3.07	2.32	2.17	.25
Small intestine					
1 (30.5 cm)	7.32	6.79	6.96	6.72	.25
2 (30.5 cm)	6.91	6.90	6.88	6.35	.24
3 (30.5 cm)	6.91	7.00	6.68	6.58	.23
4 (30.5 cm)	6.50	6.91	6.80	6.81	.17
Avg. of segments 1-4	6.91	6.90	6.83	6.61	.18
5 (61 cm)	6.53	7.02	6.95	6.80	.12
6 (61 cm)	6.57	6.86	6.88	7.03	.18
7 (61 cm)	6.90	7.60	7.08	7.16	.34
8 (61 cm)	6.80	7.65	7.22	7.38	.33
9 (61 cm)	6.84	7.91	7.52	7.63	.36
Avg. of segments 5-9	6.82	7.41	7.13	7.20	.23
10 (122 cm)	7.25	8.15	7.78	7.64	.26
11 (122 cm)	7.59	8.16	8.27	7.73	.40
12 (122 cm)	8.07	8.08	7.98	8.43	.15
Avg. of segments 10-12	7.64	8.13	8.01	7.94	.20
13 (122 cm)	8.24	8.41	8.16	8.45	.10
14 (122 cm)	8.33	8.42	8.11	8.41	.16
15 (122 cm)	8.33	8.29	8.19	8.31	.11
16 (122 cm)	8.29	8.25	8.24	8.27	.16
Avg. of segments 13-16	8.30	8.34	8.18	8.36	.09
Final S.I. sample	8.15	8.10	8.02	8.12	.14
Cecum	6.78	6.65	6.55	6.98	.09
Large intestine	7.40	7.57	7.41	7.59	.24
Rectum	7.12	6.86	7.19	6.89	.19

[a]Standard error of the treatment means. The differences among treatment means on the same line were not statistically significant.

in the proximal portion of the small intestine was much higher than that observed by Hogan and Phillipson (1960) and Phillipson and Storry (1965).

Young et al. (1966) suggested that the ruminant's ability to tolerate wide calcium to phosphorus ratios could be associated with the relatively low pH observed in the proximal portion of the small intestine. Hogan and Phillipson (1960) observed that the pH of digesta in the sheep's duodenum varied from 1.6 to 3.4. Though not stating the diet utilized, Phillipson and Storry (1965) reported that the pH of the digesta in the duodenum and jejunum ranged from 2.5 to 4.5. Storry (1961a) noted that the pH of the digesta in the proximal section (approximately 274 cm) of the small intestine of sheep ranged from 5.2 to 6.2 when the diets utilized ranged from spring grass to hay and concentrates.

According to Sisson and Grossman (1953), the small intestine of a sheep is about 24 m long. Duodenal glands exist for a distance of 60 to 70 cm beyond the pylorus, and the pancreatic and bile ducts combine to form a common duct which opens into the duodenum about 30 cm from the pylorus. When Phillipson and Storry (1965) placed various solutions including abomasal liquor in a duodenal loop which included the common bile and pancreatic duct, it was found that the pH increased from an initial value of about 2.8 to a final value ranging from 6.2 to 6.7.

Thus, there appears to be some question relative to the pH of digesta in the proximal small intestine of the ruminant. It appears that the low pH reported by Hogan and Phillipson (1960) and Phillipson and Storry (1965) may not exist posterior to the entrance of the common bile and pancreatic duct.

Results of the present experiment may have been a function of the

purified diet, however, they do suggest that the pH of the digesta in the proximal portion of the small intestine may not be low. These data indicate the need for further study to verify the pH which exists in the proximal portion of the small intestine. These data also indicate that the dietary level of calcium has little or no effect upon the pH of digesta in the alimentary tract of lambs when a purified diet is fed.

Slaughter Information, Organ Weights, Skeletal Measurements and Levels of Tissue Minerals

Considering the limited number of lambs that were sacrificed and evaluated, the carcass data and levels of tissue minerals could at best be considered only as observations. The absolute and relative slaughter data, including organ weights and skeletal measurements of the lambs, are presented in Appendix Tables XLV and XLVI, respectively. There were no statistically significant treatment differences or discernible trends.

The concentrations of various mineral elements in the lean and fat tissue of the lambs are presented in Appendix Tables XLVII and XLVIII, respectively. The levels of various mineral elements in the kidneys of the lambs sacrificed are presented in Appendix Table XLIX. No highly significant differences existed, and there were no readily apparent treatment trends. Considering the within treatment variation and the limited number of animals, a complete discussion of these data is unwarranted. The tissue analyses would perhaps have been more meaningful if the animals had been sacrificed immediately following the balance phase.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was conducted to evaluate the metabolism of calcium, phosphorus, magnesium, copper, zinc and potassium in lambs as related to the dietary level of calcium. Four levels of dietary calcium were evaluated in a completely randomized experimental design with five lambs per treatment. Purified diets containing 0.58, 1.60, 2.79 and 4.46% calcium were studied during a 231-day experimental period consisting of two phases: an 84-day balance phase followed by a 147-day growth phase. During the balance phase, the complete balance of calcium, phosphorus, magnesium, copper and zinc was determined. Wool and blood samples were obtained initially and at 21-day intervals during the entire 231-day study. Upon termination of the growth phase, three lambs were selected at random from each treatment group to study the effect of dietary calcium level upon the pH of digesta in the alimentary tract.

Though no statistically significant differences existed, the best overall growth was achieved by the lambs receiving 1.60% dietary calcium followed by those receiving 0.58, 2.79 and 4.46%, respectively. After adjusting for initial differences in serum alkaline phosphatase activity no treatment effects were apparent. No statistically significant treatment differences were observed in the hematocrit and hemoglobin concentrations. As the quantity of calcium ingested increased, the amount excreted via the feces and the amount retained increased significantly.

The amount of calcium ingested did not appear to influence the excretion and balance of phosphorus, magnesium and copper.

The dietary level of calcium appeared to have no noticeable effect upon serum levels of calcium, phosphorus, zinc and potassium. Though all serum magnesium levels were within a normal range, the concentration of serum magnesium tended to decrease as the level of dietary calcium increased. The serum copper levels of the lambs fed the highest level of calcium were significantly lower than the other treatment levels during the terminal 12 weeks on experiment. Serum levels of zinc and potassium were not influenced by the dietary level of calcium.

The concentrations of magnesium, copper and zinc in the clean dry wool samples did not appear to be influenced by the dietary level of calcium. Although the concentration of calcium in the wool was somewhat variable with time, as the level of dietary calcium increased, the concentration of calcium in the wool increased. The concentrations of phosphorus and potassium in the wool were erratic across time and the treatment effects were irregular. Considering the variation observed within treatments and with time for the elements evaluated, the value of using wool concentration as a means of monitoring mineral element status of lambs could certainly be questioned.

The dietary level of calcium had no effect upon the pH of the digesta from various segments of the alimentary tract of the anesthetized lambs.

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APPENDICES

APPENDIX A

TABLE IV

THE SOURCES OF VARIATION AND DEGREES OF FREEDOM ASSOCIATED WITH THE ANALYSES OF VARIANCE UTILIZED IN THE ANALYSIS OF DATA

Analysis of Variance			
No.	Source of Variation	D. F.[a]	Data Analyzed
1			
	Total	215	Lamb weight
	Trt.	3	Hemoglobin concentration
	Animals in trt. (exp. error)	14	Hematocrit
	Period	11	
	Period x trt.	33	
	Period x animals in trt.	154	
2			
	Total	197	Lamb weight change
	Trt.	3	Average daily gain
	Animals in trt. (exp. error)	14	Feed consumption
	Period	10	Water consumption
	Period x trt.	30	Percent wool ash
	Period x animals in trt.	140	
3			
	Total	179	Serum alkaline phosphatase activity
	Trt.	3	
	Animals in trt. (exp. error)	14	
	Period	9	
	Period x trt.	27	
	Period x animals in trt.	126	
4			
	Total	71	Ingested Ca, P, Mg, Cu and Zn
	Trt.	3	
	Animals in trt. (exp. error)	14	
	Period	3	
	Period x trt.	9	
	Period x animals in trt.	42	

TABLE IV (continued)

Analysis of Variance			
No.	Source of Variation	D. F.[a]	Data Analyzed
5			
	Total	431	Serum Ca, P, Mg, Cu, Zn and K
	Trt.	3	
	Animals in trt. (exp. error)	14	
	Period	11	
	Period x trt.	33	
	Period x animals in trt.	154	
	Duplicates in animals in period	216	
6			
	Total	431	Fecal Ca, P, Mg, Cu and Zn
	Trt.	3	
	Animals in trt. (exp. error)	14	
	Period	3	
	Period x trt.	9	
	Period x animals in trt.	42	
	Samples in animal in period	144	
	Duplicates in samples in animal in period	216	
7			
	Total	143	Urinary Ca, P, Mg and Zn
	Trt.	3	
	Animals in trt. (exp. error)	14	
	Period	3	
	Period x trt.	9	
	Period x animals in trt.	42	
	Duplicates in animal in period	72	
8			
	Total	395	Wool Ca, P, Mg, Cu, Zn and K
	Trt.	3	
	Animals in trt. (exp. error)	14	
	Period	10	
	Period x trt.	30	
	Period x animals in trt.	140	
	Duplicates in animals in period	198	

TABLE IV (continued)

Analysis of Variance			
No.	Source of Variation	D. F.[a]	Data Analyzed
9			
	Total	47	All tissue analyses
	Trt.	3	
	Animals in trt. (exp. error)	8	
	Samples in animal	12	
	Duplicates in samples in animal	24	
10			
	Total	11	Alimentary tract pH
	Trt.	3	Slaughter and carcass data
	Animals in trt. (exp. error)	8	

[a]Degrees of freedom.

APPENDIX B

TABLE V

ABSOLUTE WEIGHTS[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment:				
0	18.1	16.7	18.1	18.4
3	19.0	20.2	19.0	18.5
6	20.2	21.5	20.2	19.2
9	21.1	23.9	22.5	20.2
12	23.0	26.3	24.1	21.3
15	25.3	29.0	25.1	22.5
18	28.6	31.7	26.7	24.6
21	30.9	34.3	29.0	26.8
24	33.2	36.3	30.5	28.9
27	36.3	38.7	33.2	31.4
30	38.8	40.9	35.2	33.1
33	41.7	42.7	37.7	34.7
Overall mean[b]	28.0	30.2	26.8	25.0
SE[c]	2.36	1.83	1.83	1.83

[a]Kg.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE VI

WEIGHT CHANGE[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
3	0.90	3.50	0.96	0.10
6	2.03	4.78	2.14	0.74
9	2.93	7.18	4.44	1.76
12	4.90	9.62	6.08	4.00
15	7.13	12.28	7.06	4.10
18	10.50	14.96	8.10	6.30
21	12.77	17.60	10.98	8.38
24	15.07	19.54	12.46	10.52
27	18.13	21.98	15.10	12.94
30	20.67	24.22	17.10	14.68
33	23.60	25.98	19.60	16.30
Overall mean[b]	10.78	14.69	9.46	7.26
SE[c]	2.60	2.01	2.01	2.01

[a]Kg, each value represents the difference between the initial weight and the weight at the given times.

[b]Level of significance among overall means was approximately 0.296.

[c]Standard errors apply only to the overall means.

TABLE VII

AVERAGE DAILY GAIN[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	42.7	166.6	45.6	4.8
2	54.0	61.2	56.2	30.4
3	43.0	114.2	109.6	48.6
4	93.7	116.2	78.0	53.2
5	106.7	126.6	46.4	58.0
6	161.7	130.6	77.6	101.8
7	106.3	122.4	109.2	101.8
8	109.7	92.2	70.4	101.8
9	146.0	116.2	125.8	115.4
10	120.7	106.6	95.2	82.4
11	142.0	85.4	122.0	79.6
Overall mean[b]	102.4	112.6	85.1	70.7
SE[c]	17.1	13.3	13.3	13.3

[a]Gm/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE VIII

AVERAGE DAILY FEED CONSUMPTION[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	598.7	793.0	626.9	608.9
2	681.6	847.4	763.6	662.2
3	797.3	1019.1	988.4	742.2
4	896.2	1150.0	986.7	847.1
5	963.7	1313.4	1011.5	890.6
6	1200.2	1346.5	1099.3	983.5
7	1305.1	1383.7	1199.6	1199.2
8	1198.4	1315.4	1190.2	1170.0
9	1390.8	1302.7	1235.8	1254.6
10	1372.7	1370.2	1355.1	1217.9
11	1404.1	1270.5	1349.0	1241.6
Overall mean[b]	1073.5	1192.0	1073.3	983.4
SE[c]	111.1	86.0	86.0	86.0

[a] Gm/day.

[b] No significant differences exist among the overall means.

[c] Standard errors apply only to the overall means.

TABLE IX

AVERAGE DAILY WATER CONSUMPTION[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	1059	1516	1164	1199
2	1138	1514	1315	1240
3	1337	1823	1580	1418
4	1678	2337	2094	1943
5	1974	2613	2810	2365
6	2482	2980	3053	2792
7	2942	3301	3551	3390
8	3224	3643	4122	3965
9	4323	4328	4370	4118
10	4454	4095	4351	3511
11	4396	3570	3690	2910
Overall mean[b]	2637	2883	2918	2623
SE[c]	378	292	292	292

[a]Ml/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

APPENDIX C

TABLE X

SERUM ALKALINE PHOSPHATASE ACTIVITY[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	4.975	3.197	2.891	2.798
3	5.768	4.538	2.723	3.096
6	6.357	4.044	2.600	2.527
9	5.863	4.933	3.313	3.788
12	4.917	5.889	3.281	3.447
15	4.952	5.458	2.430	2.819
18	5.611	4.157	2.356	2.571
21	3.377	5.304	3.147	2.896
24	5.502	5.138	3.591	3.343
27				
30				
33	4.683	4.005	2.634	3.141
Overall mean[b]	5.200	4.666	2.897	3.042
SE[c]	0.734	0.569	0.569	0.569

[a] μM p-nitrophenol liberated/hr/ml serum.

[b] The differences among overall treatment means were significant ($P < .05$).

[c] Standard errors apply only to overall means.

TABLE XI

CONCENTRATION[a] OF HEMOGLOBIN IN THE BLOOD OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	15.76	14.51	15.48	15.16
3	14.27	12.66	14.84	14.77
6	14.37	12.16	13.85	13.99
9	13.44	10.82	11.94	11.33
12	13.16	12.24	13.38	12.65
15	13.50	13.54	13.13	12.51
18	13.81	12.97	12.48	11.96
21	11.43	12.05	12.06	11.21
24	12.89	12.72	13.19	11.73
27	13.84	11.91	13.50	12.03
30	13.90	12.07	13.25	11.07
33	12.72	11.46	11.51	10.21
Overall mean[b]	13.59	12.43	13.22	12.39
SE[c]	0.49	0.38	0.38	0.38

[a] Gm/100 ml.

[b] No significant differences exist among the overall means.

[c] Standard errors apply only to the overall means.

TABLE XII

HEMATOCRIT[a] IN THE BLOOD OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	47.67	43.70	46.25	43.55
3	41.42	37.50	43.30	41.85
6	39.36	36.45	41.80	41.00
9	36.00	34.90	36.70	36.50
12	35.67	34.40	36.80	35.15
15	36.75	37.60	36.15	35.55
18	37.08	37.25	35.75	34.00
21	35.50	36.75	37.50	35.90
24	36.17	36.50	39.00	34.60
27	36.58	33.90	37.70	33.95
30	38.00	33.55	35.65	32.70
33	37.58	34.35	34.90	30.85
Overall mean[b]	38.15	36.40	38.46	36.30
SE[c]	1.19	0.92	0.92	0.92

[a]Percent packed cell volume.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

APPENDIX D

TABLE XIII

CALCIUM INGESTED[a] BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	3.464	12.691	17.481	27.183
2	3.943	13.563	21.292	29.565
3	4.612	16.311	27.562	33.137
4	5.185	18.406	27.511	37.818
Overall mean[b]	4.301	15.243	23.461	31.926
SE[c]	3.368	2.609	2.609	2.609

[a]Gm/day.

[b]The differences among overall treatment means were significant ($P < .01$).

[c]Standard errors apply only to the overall means.

TABLE XIV

CALCIUM EXCRETED[a] IN THE FECES BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	2.966	8.812	11.715	15.431
2	3.678	11.088	16.775	20.808
3	4.205	11.753	22.906	23.736
4	4.277	14.435	21.040	26.403
Overall mean[b]	3.781	11.522	18.108	21.594
SE[c]	5.739	4.446	4.446	4.446

[a]Gm/day.

[b]The differences among overall treatment means were significant ($P < .01$).

[c]Standard errors apply only to the overall means.

TABLE XV

CALCIUM EXCRETED[a] IN THE URINE BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	4.858	6.681	4.716	3.062
2	2.950	3.892	5.169	3.771
3	3.180	3.976	4.140	3.175
4	3.411	3.949	5.006	5.407
Overall mean[b]	3.600	4.625	4.758	3.854
SE[c]	1.453	1.125	1.125	1.125

[a]Mg/day.

[b]No significant differences exist among overall means.

[c]Standard errors apply only to the overall means.

TABLE XVI

THE CALCIUM BALANCE[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	0.493	3.872	5.761	11.749
2	0.284	2.474	4.512	8.747
3	0.404	4.554	4.651	9.398
4	0.904	3.967	6.466	11.410
Overall mean[b]	0.457	3.717	5.347	10.326
SE[c]	1.158	0.897	0.897	0.897

[a]Gm/day.

[b]The differences among overall treatment means were significant ($P < .05$).

[c]Standard errors apply only to the overall means.

TABLE XVII

PHOSPHORUS INGESTED[a] BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	2.249	2.801	2.231	2.170
2	2.561	2.994	2.717	2.361
3	2.995	3.601	3.517	2.646
4	3.367	4.063	3.510	3.020
Overall mean[b]	2.793	3.365	2.994	2.549
SE[c]	0.492	0.381	0.381	0.381

[a]Gm/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XVIII

PHOSPHORUS EXCRETED[a] IN THE FECES BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	1.091	1.368	1.214	1.173
2	1.273	1.616	1.663	1.422
3	1.486	1.504	1.915	1.614
4	1.669	2.343	2.290	1.896
Overall mean[b]	1.380	1.708	1.770	1.526
SE[c]	0.780	0.604	0.604	0.604

[a]Gm/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XIX

PHOSPHORUS EXCRETED[a] IN THE URINE BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	110.0	176.3	137.2	92.4
2	135.8	358.6	252.8	163.0
3	336.1	616.6	402.2	310.8
4	246.8	501.5	461.1	344.8
Overall mean[b]	207.2	413.2	313.3	227.8
SE[c]	159.9	123.9	123.9	123.9

[a]Mg/day.

[b]No significant differences exist among overall means.

[c]Standard errors apply only to the overall means.

TABLE XX

THE PHOSPHORUS BALANCE[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	1.058	1.256	0.872	0.863
2	1.151	1.019	0.801	0.776
3	1.173	1.480	1.200	0.721
4	1.451	1.218	0.903	0.701
Overall mean[b]	1.208	1.243	0.944	0.765
SE[c]	0.250	0.194	0.194	0.194

[a]Gm/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXI

MAGNESIUM INGESTED[a] BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	613	814	690	695
2	698	870	906	755
3	816	1046	1087	847
4	918	1181	1085	967
Overall mean[b]	761	978	942	816
SE[c]	144	112	112	112

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXII

MAGNESIUM EXCRETED[a] IN THE FECES BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	336.6	494.6	422.7	431.7
2	333.2	528.7	493.1	469.3
3	412.1	531.7	598.6	487.6
4	438.2	640.5	551.3	536.5
Overall mean[b]	380.0	548.9	516.4	481.3
SE[c]	184.7	143.1	143.1	143.1

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXIII

MAGNESIUM EXCRETED[a] IN THE URINE BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	0.442	0.111	0.092	0.848
2	0.465	0.193	0.080	0.118
3	0.285	0.246	0.163	0.362
4	0.243	0.212	0.279	0.413
Overall mean[b]	0.359	0.190	0.153	0.435
SE[c]	0.131	0.102	0.102	0.102

[a]Mg/day.

[b]Level of significance among overall means was approximately 0.10.

[c]Standard errors apply only to the overall means.

TABLE XXIV

THE MAGNESIUM BALANCE[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	276.3	319.7	304.4	291.2
2	264.3	341.3	413.2	285.9
3	403.9	514.4	488.4	358.8
4	478.5	540.5	533.8	429.6
Overall mean[b]	380.8	429.0	435.0	341.4
SE[c]	73.9	57.3	57.3	57.3

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXV

COPPER INGESTED[a] BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	7.872	9.034	8.610	9.019
2	8.961	9.654	10.488	9.809
3	10.483	11.610	13.355	10.994
4	11.783	13.101	13.551	12.547
Overall mean[b]	9.775	10.850	11.501	10.592
SE[c]	1.831	1.418	1.418	1.418

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXVI

COPPER EXCRETED[a] IN THE FECES BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	5.331	7.244	6.587	6.851
2	6.381	9.556	9.492	7.750
3	6.794	9.108	10.125	8.702
4	8.266	12.378	10.284	9.476
Overall mean[b]	6.693	9.571	9.122	8.195
SE[c]	3.222	2.495	2.495	2.495

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXVII

THE COPPER BALANCE[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	2.541	1.774	2.023	2.169
2	2.581	0.098	0.996	2.059
3	3.689	2.503	3.229	2.292
4	3.517	0.723	3.267	3.072
Overall mean[b]	3.082	1.274	2.379	2.398
SE[c]	0.639	0.495	0.495	0.495

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXVIII

ZINC INGESTED[a] BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	32.312	45.078	41.059	30.768
2	36.784	48.174	50.003	33.463
3	43.029	57.936	64.735	37.506
4	48.366	65.375	64.616	42.805
Overall mean[b]	40.122	54.141	55.103	36.135
SE[c]	7.697	5.962	5.962	5.962

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXIX

ZINC EXCRETED[a] IN THE FECES BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	31.982	42.508	32.716	32.495
2	35.945	46.599	42.099	37.575
3	39.437	47.453	54.816	42.417
4	44.630	60.056	49.308	43.680
Overall mean[b]	37.999	49.154	44.735	39.042
SE[c]	17.052	13.209	13.209	13.209

[a]Mg/day.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XXX

ZINC EXCRETED[a] IN THE URINE BY THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	0.214	0.189	0.189	0.129
2	0.166	0.171	0.184	0.130
3	0.141	0.152	0.186	0.107
4	0.296	0.146	0.121	0.136
Overall mean[b]	0.204	0.165	0.170	0.126
SE[c]	0.046	0.035	0.035	0.035

[a]Mg/day.

[b]No significant differences exist among overall means.

[c]Standard errors apply only to the overall means.

TABLE XXXI

THE ZINC BALANCE[a] OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Period				
1	0.116	2.381	8.194	-1.857
2	0.673	1.404	7.721	-4.242
3	3.451	10.331	9.771	-5.018
4	3.440	5.260	15.187	-1.011
Overall mean[b]	1.920	4.844	10.219	-3.032
SE[c]	1.695	1.313	1.313	1.313

[a]Mg/day.

[b]The differences among overall treatment means were significant ($P < .01$).

[c]Standard errors apply only to the overall means.

APPENDIX E

TABLE XXXII

CONCENTRATION[a] OF CALCIUM IN THE SERUM OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	11.01	11.12	11.65	11.57
3	11.02	11.03	11.43	10.89
6	10.63	10.86	11.40	10.47
9	9.66	10.59	10.66	10.39
12	9.61	10.61	10.26	10.14
15	10.16	10.66	10.13	9.91
18	10.09	10.43	10.40	10.03
21	10.09	10.43	10.56	10.28
24	10.20	10.64	10.27	10.10
27	10.18	10.42	10.26	10.14
30	10.37	10.49	10.08	9.96
33	9.80	10.32	10.11	9.37
Overall mean[b]	10.24	10.63	10.60	10.27
SE[c]	0.43	0.33	0.33	0.33

[a] Mg/100 ml.

[b] No significant differences exist among the overall means.

[c] Standard errors apply only to the overall means.

TABLE XXXIII

CONCENTRATION[a] OF PHOSPHORUS IN THE SERUM OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	6.66	5.80	6.58	6.01
3	5.20	5.87	5.68	5.48
6	7.46	6.67	6.23	5.87
9	7.09	5.93	5.98	6.75
12	6.95	5.97	4.99	5.36
15	5.01	5.76	4.53	4.56
18	6.70	5.03	4.20	4.12
21	5.61	4.42	4.17	4.47
24	5.27	4.79	4.00	3.94
27	5.36	4.86	4.24	4.34
30	6.52	5.77	4.97	5.48
33	7.57	6.56	5.54	7.12
Overall mean[b]	6.29	5.62	5.09	5.29
SE[c]	0.55	0.42	0.42	0.42

[a]Mg/100 ml.

[b]Level of significance among overall means was approximately 0.294.

[c]Standard errors apply only to the overall means.

TABLE XXXIV

CONCENTRATION[a] OF MAGNESIUM IN THE SERUM OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	2.467	2.434	2.428	2.514
3	2.738	2.466	2.332	2.492
6	2.465	2.492	2.420	2.291
9	2.677	2.578	2.525	2.402
12	2.691	2.851	2.565	2.596
15	2.952	2.715	2.484	2.486
18	2.832	2.674	2.519	2.386
21	2.872	2.586	2.550	2.671
24	2.919	2.597	2.526	2.598
27	2.766	2.649	2.721	2.627
30	2.991	2.760	2.806	2.713
33	3.085	2.686	2.825	2.709
Overall mean[b]	2.788	2.624	2.558	2.540
SE[c]	0.095	0.074	0.074	0.074

[a]Mg/100 ml.

[b]Level of significance among overall means was approximately 0.11.

[c]Standard errors apply only to overall means.

TABLE XXXV

CONCENTRATION[a] OF COPPER IN THE SERUM OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	98.0	99.5	87.2	86.6
3	101.8	110.5	109.3	101.1
6	133.5	131.9	107.4	113.4
9	127.1	147.2	121.6	101.7
12	104.1	88.2	91.8	87.8
15	99.6	94.9	103.7	98.3
18	98.7	102.9	99.8	95.3
21	89.7	85.3	92.6	75.0
24	89.6	89.9	94.7	72.6
27	111.1	83.2	93.6	69.3
30	108.2	96.4	91.2	66.3
33	95.7	115.7	104.9	66.1
Overall mean[b]	104.8	103.8	99.8	86.1
SE[c]	6.96	5.39	5.39	5.39

[a] μg/100 ml.

[b] The differences among overall treatment means were significant ($P < .05$).

[c] Standard errors apply only to the overall means.

TABLE XXXVI

CONCENTRATION[a] OF ZINC IN THE SERUM OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	96.8	92.9	88.1	90.5
3	80.6	85.6	84.3	80.8
6	73.7	94.9	89.6	76.8
9	71.1	78.9	83.3	79.7
12	70.3	80.3	78.3	77.2
15	70.0	93.5	82.1	76.1
18	70.8	87.5	69.9	72.5
21	71.2	81.3	67.6	69.1
24	99.4	92.4	77.8	87.0
27	91.5	83.3	73.8	81.4
30	87.2	84.9	76.1	80.8
33	78.9	84.3	62.9	73.5
Overall mean[b]	80.1	86.7	77.8	78.8
SE[c]	6.73	5.21	5.21	5.21

[a] μg/100 ml.

[b] No significant differences exist among the overall means.

[c] Standard errors apply only to the overall means.

TABLE XXXVII

CONCENTRATION[a] OF POTASSIUM IN THE SERUM OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	23.65	21.97	22.92	23.68
3	21.13	22.22	23.52	23.75
6	24.66	25.08	25.63	24.48
9	25.87	25.80	25.76	26.53
12	24.86	22.55	23.09	22.82
15	25.76	24.70	23.49	22.84
18	23.64	23.46	22.70	21.96
21	22.19	21.44	21.89	21.41
24	23.76	21.52	21.03	21.16
27	22.52	21.16	22.08	21.13
30	23.30	22.78	21.88	22.02
33	21.96	21.67	20.84	20.89
Overall mean[b]	23.61	22.86	22.90	22.72
SE[c]	0.71	0.55	0.55	0.55

[a]Mg/100 ml.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

APPENDIX F

TABLE XXXVIII

ASH CONTENT[a] OF THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, % A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	0.169	0.235	0.215	0.227
3	0.126	0.165	0.225	0.168
6	0.397	0.708	0.780	0.741
9	0.231	0.323	0.302	0.425
12	0.243	0.336	0.516	0.504
15	0.254	0.300	0.529	0.651
18	0.199	0.228	0.289	0.427
21				
24	0.346	0.231	0.270	0.286
27	0.370	0.425	0.345	0.373
30	0.200	0.267	0.314	0.227
33	0.210	0.185	0.279	0.265
Overall mean[b]	0.250	0.309	0.369	0.390
SE[c]	0.045	0.035	0.035	0.035

[a]Percent.

[b]Level of significance among the overall means was approximately 0.20.

[c]Standard errors apply only to the overall means.

TABLE XXXIX

CONCENTRATION[a] OF CALCIUM IN THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	273	307	330	277
3	213	272	544	382
6	594	1392	2046	1719
9	546	765	744	1114
12	593	1006	1633	1637
15	442	771	1567	1671
18	442	539	723	1253
21				
24	473	456	688	809
27	520	486	708	850
30	338	529	947	642
33	319	458	619	693
Overall mean[b]	432	635	959	1005
SE[c]	206	160	160	160

[a]Parts per million.

[b]The differences among overall treatment means were significant ($P < .05$).

[c]Standard errors apply only to the overall means.

TABLE XL

CONCENTRATION[a] OF PHOSPHORUS IN THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

	Dietary calcium level, %			
Item	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	143	156	151	142
3	154	170	173	142
6	173	307	417	284
9	253	330	295	455
12	227	315	519	404
15	206	268	371	468
18	220	189	189	440
21				
24	138	188	238	333
27	134	151	273	382
30	128	163	238	303
33	155	212	227	325
Overall mean[b]	175	223	281	334
SE[c]	77	59	59	59

[a]Parts per million.

[b]Level of significance among the overall means was approximately 0.30.

[c]Standard errors apply only to the overall means.

TABLE XLI

CONCENTRATION[a] OF MAGNESIUM IN THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	51.6	51.9	52.5	51.3
3	37.4	37.7	47.6	34.7
6	87.8	141.6	118.7	95.7
9	40.8	63.6	49.1	53.3
12	86.8	85.1	89.3	66.3
15	72.2	71.8	105.4	80.8
18	72.2	66.4	77.6	75.0
21				
24	131.3	112.0	134.6	114.0
27	145.3	124.7	147.4	113.1
30	89.6	127.1	132.9	75.8
33	69.6	69.6	86.9	80.9
Overall mean[b]	80.4	86.5	94.7	76.4
SE[c]	23.3	18.1	18.1	18.1

[a]Parts per million.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XLII

CONCENTRATION[a] OF COPPER IN THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	27.4	36.4	36.5	30.1
3	68.3	87.4	72.2	65.7
6	28.5	58.4	44.2	72.1
9	103.9	91.7	75.5	213.0
12	74.1	67.4	92.3	72.6
15	104.8	86.6	107.9	98.4
18	90.7	82.7	71.9	91.4
21				
24	65.2	66.5	85.9	83.5
27	141.0	53.3	60.1	155.3
30	172.9	132.0	95.9	106.4
33	89.6	53.1	152.3	82.9
Overall mean[b]	87.9	74.1	81.3	97.4
SE[c]	13.6	10.6	10.6	10.6

[a]Parts per million.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XLIII

CONCENTRATION[a] OF ZINC IN THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, % A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	98.9	96.3	109.5	99.7
3	127.9	129.6	137.0	120.6
6	113.0	132.9	161.5	143.2
9	138.4	173.1	138.9	148.9
12	143.8	147.6	166.9	148.4
15	134.2	143.8	154.9	165.3
18	134.6	125.4	105.4	125.1
21				
24	103.0	121.5	138.2	147.2
27	111.2	118.3	142.7	146.6
30	92.9	127.5	133.6	130.6
33	118.5	124.6	133.5	138.1
Overall mean[b]	119.7	130.9	138.4	137.6
SE[c]	11.2	8.7	8.7	8.7

[a]Parts per million.

[b]No significant differences exist among the overall means.

[c]Standard errors apply only to the overall means.

TABLE XLIV

CONCENTRATION[a] OF POTASSIUM IN THE WOOL OF THE LAMBS FED THE VARIOUS LEVELS OF CALCIUM

Item	Dietary calcium level, %			
	A 0.58	B 1.60	C 2.79	D 4.46
No. of lambs	3	5	5	5
Weeks on experiment				
0	35.0	14.3	24.4	18.9
3	16.7	14.1	7.6	8.9
6	79.3	48.5	29.3	24.5
9	13.8	23.1	12.0	7.6
12	8.9	13.3	11.6	12.4
15	18.5	9.9	6.5	10.4
18	18.5	11.5	18.2	16.5
21				
24	82.0	45.0	39.1	20.7
27	82.6	86.0	53.3	42.1
30	57.8	63.9	13.7	11.5
33	57.2	30.1	30.0	19.8
Overall mean[b]	42.8	32.7	22.3	17.6
SE[c]	7.0	5.4	5.4	5.4

[a]Parts per million.

[b]The differences among overall treatment means were significant ($P < .01$).

[c]Standard errors apply only to the overall means.

APPENDIX G

TABLE XLV

ABSOLUTE AND RELATIVE SLAUGHTER INFORMATION AND ORGAN WEIGHTS OF THE LAMBS SACRIFICED FOLLOWING TERMINATION OF THE GROWTH PHASE

	Dietary calcium level, %				
Item	A 0.58	B 1.60	C 2.79	D 4.46	SE[a,b]
No. of lambs	3	3	3	3	
Slaughter wt, kg	41.9	45.0	41.0	34.5	3.28
Carcass wt, kg	20.6	24.6	22.2	16.5	2.03
Dressing percent	49.4	54.2	54.2	48.0	2.05
Heart wt, gm	176.0	170.0	170.0	156.3	15.4
Liver wt, gm	513.7	680.7	446.3	444.0	120.2
Kidney wt, gm	91.0	91.7	83.3	79.3	7.2
Heart wt, gm/kg carcass	8.52	7.09	7.65	9.41	0.53
Liver wt, gm/kg carcass	24.90	26.39	20.05	26.55	2.73
Kidney wt, gm/kg carcass	4.41	3.81	3.77	4.75	0.22

[a]Standard error of the treatment means.

[b]No significant differences exist among the means on the same line.

TABLE XLVI

ABSOLUTE AND RELATIVE SKELETAL MEASUREMENTS OF THE LAMBS SACRIFICED FOLLOWING TERMINATION OF THE GROWTH PHASE

Item	Dietary calcium level, % A 0.58	B 1.60	C 2.79	D 4.46	SE[a,b]
No. of lambs	3	3	3	3	
Right femur wt, gm	113.0	125.2	109.2	98.0	10.7
Right tibia wt, gm	87.7	98.0	86.1	74.0	7.5
Right femur wt, gm/kg carcass	5.46	5.15	4.92	5.95	0.34
Right tibia wt, gm/kg carcass	4.24	4.04	3.89	4.52	0.28
Right femur length, cm	17.14	17.24	16.76	16.56	0.51
Right tibia length, cm	17.64	18.16	17.45	17.47	0.50
Right femur length, cm/kg carcass	0.83	0.72	0.76	1.02	0.06
Right tibia length, cm/kg carcass	0.85	0.76	0.79	1.08	0.07
Right femur specific gravity	1.266	1.285	1.283	1.247	0.019
Right tibia specific gravity	1.317	1.331	1.336	1.305	0.019
Length of body, cm	61.38	60.96	61.13	59.52	1.40
Length of leg, cm	49.36	50.12	48.18	48.60	1.33
Depth of body, cm	19.73	19.31	18.71	19.73	0.53
Length of body, cm/kg carcass	2.97	2.55	2.76	3.68	0.23
Length of leg, cm/kg carcass	2.39	2.10	2.18	3.00	0.18
Depth of body, cm/kg carcass	0.96	0.81	0.85	1.22	0.09

[a]Standard error of the treatment means.

[b]No significant differences exist among the means on the same line.

APPENDIX H

TABLE XLVII

CONCENTRATION OF VARIOUS MINERALS IN THE LEAN TISSUE OF THE LAMBS SACRIFICED FOLLOWING TERMINATION OF THE GROWTH PHASE

	Dietary calcium level, %					
Item	A 0.58	B 1.60	C 2.79	D 4.46	SE[a]	LS[b]
No. of lambs	3	3	3	3		
Wet basis						
Ca, ppm	82	79	109	64	47.8	NS
P, ppm	2250	2410	2242	2131	145.4	.31
Mg, ppm	237	232	228	220	14.1	NS
Cu, ppm	3.7	3.3	3.6	2.6	0.95	NS
Zn, ppm	26.2	25.2	25.0	25.1	2.9	NS
K, ppm	2690	2659	2600	2976	213.5	.31
Dry basis						
Ca, ppm	308	306	413	272	182.0	NS
P, ppm	8391	9020	8670	7862	535.0	.16
Mg, ppm	876	868	880	813	58.3	NS
Cu, ppm	13.7	12.5	13.8	9.6	3.5	NS
Zn, ppm	97.6	94.2	96.9	92.7	8.8	NS
K, ppm	10058	9940	10057	10970	800	NS

[a]Standard error of the treatment means.

[b]Level of significance, NS = not significant.

TABLE XLVIII

CONCENTRATION OF VARIOUS MINERALS IN THE FAT TISSUE OF THE LAMBS SACRIFICED FOLLOWING TERMINATION OF THE GROWTH PHASE

	Dietary calcium level, %					
Item	A 0.57	B 1.60	C 2.79	D 4.46	SE[a]	LS[b]
No. of lambs	3	3	3	3		
Wet basis						
Ca, ppm	20.5	13.5	14.3	19.6	4.87	.38
P, ppm	119.9	94.8	95.2	131.9	22.12	.23
Mg, ppm	8.0	6.3	6.5	8.6	1.43	.32
Cu, ppm	1.4	2.4	1.7	0.7	1.46	NS
Zn, ppm	0.65	0.58	0.66	0.68	0.10	NS
K, ppm	33.4	25.6	33.2	40.9	15.14	NS
Dry basis						
Ca, ppm	21.0	13.8	14.7	20.8	5.26	.38
P, ppm	123.0	97.5	97.5	140.4	24.02	.19
Mg, ppm	8.2	6.5	6.6	9.2	1.58	.26
Cu, ppm	1.5	2.5	1.7	0.8	1.50	NS
Zn, ppm	0.67	0.59	0.68	0.72	0.11	NS
K, ppm	34.4	26.3	34.0	43.7	16.07	NS

[a]Standard error of the treatment means.

[b]Level of significance, NS = not significant.

TABLE XLIX

CONCENTRATION OF VARIOUS MINERALS IN THE KIDNEYS OF THE LAMBS SACRIFICED FOLLOWING TERMINATION OF THE GROWTH PHASE

Item	Dietary calcium level, %					
	A 0.58	B 1.60	C 2.79	D 4.46	SE[a]	LS[b]
No. of lambs	3	3	3	3		
Wet basis						
Ca, ppm	211	213	176	187	52.7	NS
P, mg/100 g	218	218	211	221	19.7	NS
Mg, ppm	174	197	163	182	15.9	.18
Cu, ppm	7.8	7.7	6.1	7.8	4.8	NS
Zn, ppm	18.5	19.5	18.0	16.6	2.7	NS
K, ppm	1122	1346	914	963	423.7	NS
Dry basis						
Ca, ppm	1056	1047	836	975	226.5	NS
P, mg/100 g	1099	1076	1008	1166	80.2	.26
Mg, ppm	880	1013	782	967	102.8	.11
Cu, ppm	39.9	38.3	28.9	40.2	24.1	NS
Zn, ppm	92.7	95.9	85.7	59.0	31.7	NS
K, ppm	5673	6665	4263	5171	2175.9	NS
Total kidney content						
Ca, mg	19.1	19.2	14.5	14.1	2.6	.09
P, mg	198.8	199.5	174.9	174.8	28.4	NS
Mg, mg	16.0	18.1	13.6	14.5	2.9	NS
Cu, mg	0.74	0.72	0.50	0.66	0.47	NS
Zn, mg	1.68	1.76	1.49	1.28	0.25	.19
K, mg	102.5	124.8	73.8	79.7	41.0	NS

[a]Standard error of the treatment means.

[b]Level of significance, NS = not significant.

VITA

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John Alfred Stuedemann

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE METABOLISM OF CALCIUM, PHOSPHORUS, MAGNESIUM, COPPER, ZINC AND POTASSIUM IN LAMBS AS RELATED TO DIETARY CALCIUM LEVEL

Major Field: Animal Nutrition

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